PARASITOLOGY

CAMBRIDGE UNIVERSITY PRESS
LONDON: FETTER LANE, E.C. 4

LONDON: H. K. LEWIS AND CO., LTD., 136 Gower Street, W.C. 1
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
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PARASITOLOGY

EDITED BY

GEORGE H. F. NUTTALL, F.R.S.

QUICK PROFESSOR OF BIOLOGY AND DIRECTOR OF THE MOLTENO INSTITUTE FOR RESEARCH IN PARASITOLOGY IN THE UNIVERSITY OF CAMBRIDGE

ASSISTED BY

D. KEILIN, F.R.S.

VOLUME XXII 1930

CAMBRIDGE

AT THE UNIVERSITY PRESS

1930

PRINTED IN GREAT BRITAIN

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VOLUME XXII JANUARY, 1930 No. 1

ICONOGRAPHIC STUDIES OF *PEDICULUS HUMANUS.*

BY D. KEILIN AND G. H. F. NUTTALL.

Molteno Institute for Research in Parasitology, Cambridge.

(With Plates I–XVIII.)

THE great importance of lice as parasites and vectors of disease in man led one of us (Nuttall), soon after the outbreak of the Great War, to undertake a detailed study of these insects. It was known, prior to the war (1909–14), that lice transmit typhus and relapsing fever and during the war (1917–18) it was established that they also serve as vectors of trench fever. Beginning with February 1917, a number of papers dealing with various aspects of the louse problem were published in *Parasitology* (see vols. X–XIII), the study of their structure, systematics and biology being actively pursued whilst the best methods of combating them were sought for with a view to aiding the armies in the field. Of these papers, that dealing with the structure and function of the genitalia in *Pediculus* (II, 1917) bears directly on the subjects to which this Iconography relates.

In the course of these investigations it was decided to write a monograph to be entitled *The Lice infesting Man*, and two coloured plates were prepared and printed by the prospective author (Nuttall). This accounts for the name of the author and book-title that are printed at the top of each plate. These plates, numbered I and II, after having been stored for years, are incorporated in this Iconography, the work on the monograph having long since been abandoned owing to illness and other causes that impeded the author's progress with the work. Numerous other drawings were prepared for the monograph, some by each of the authors of this Iconography.

Moreover, the present authors collaborated on certain problems: Upon encountering that of *hermaphroditism* in *Pediculus* we were able to show that this condition arises mainly when the two races (*capitis* and *corporis*) of *P. humanus* are crossed (*vide* Keilin and Nuttall, 1919). Subsequently we described the structure, function and distribution of peculiar cells found in the body cavity of *Pediculus*, which we identified as *nephrocytes* (*vide* Nuttall and Keilin, 1921). While collaborating, we accumulated further iconographic material, but owing to numerous interruptions and other engrossing occupations we have been obliged to abandon further work on *Pediculus* and in consequence have decided to publish a selection of those figures which seem to us most worthy, although they represent but "chips from the workshop."

Under the circumstances we have deemed it best to let certain illustrations speak for themselves whilst others are accompanied by more or less explanatory matter according to requirements.

We fully appreciate the fragmentary character of our Iconography, but plead in justification that it records certain anatomical features in *Pediculus* not hitherto observed and in several instances corrects misapprehensions. We hope therefore that our studies will prove helpful to future workers on the anatomy of Anoplura and other Insects.

We have not dealt with the mouth-parts of *Pediculus*, regarding which the reader is referred to the papers by Harrison (1916), Sikora (1916) and Peacock (1918). The few references to the literature that follow the plates have been selected as dealing with the anatomy of *Pediculus* and *Phthirus*; a list of exhaustive bibliographies being appended.

EXPLANATION OF PLATES I—XVIII.

All figures refer to *Pediculus humanus* race *corporis* except Pl. VII, fig. 12, of *P. humanus* race *capitis*, and Pl. XVIII, fig. 48, of *Phthirus pubis*, which is inserted for comparison with Pl. V, fig. 6, and Pl. X, fig. 23.

We may note here that the name *corporis* de Geer, 1778, has priority over *vestimenti* Nitzsch, 1818, and that the latter name should be dropped once and for all. Similarly *Phthirus* (not *Phthirius*) is the correct spelling of the generic name established by Leach, 1815. (See Nuttall, x. 1919, pp. 329–46; iii. 1920, pp. 136–54.)

Plate I.

Pediculus humanus.

Living first stage larva, dorsal aspect.

Plates I and II are lithographic reproductions of watercolour drawings made from living specimens by Mr A. J. E. Terzi on the basis of studies by Nuttall. The irregular dark areas extending down the abdomen represent the intestine and contents which are visible owing to the insects being translucent.

Plate II.

Pediculus humanus.

Living male and female, dorsal aspect.

Plate III.

Pediculus humanus.

Fig. 1. Male, dorsal aspect, in outline, giving the nomenclature of various parts of the body. *N. del.* (See Pl. V, fig. 6; Pl. X, fig. 23; and Pl. XVIII, fig. 48.)

PLATE IV.

Pediculus humanus.

Fig. 2. Dorsal view of first stage larva soon after hatching from egg. Unfed specimen. *N. del.*

Fig. 3. Dorsal view of first stage larva. Compressed specimen showing the distribution of excretory granules, probably the urates, in the fat body cells. The excretory granules are agglomerated into opaque masses within the protoplasm of the fat body cells and their distribution can be noted especially when the specimen is examined by reflected light or with the dark ground illumination. *K. del.*

PLATE V.

Pediculus humanus.

Fig. 4. Second stage larva, dorsal aspect.

Fig. 5. Third stage larva, dorsal aspect.

Fig. 6. A portion of the exuvia (thorax and abdomen) of the third stage larva viewed ventrally, showing the first three spiracles. The first, probably prothoracic, highly developed functional spiracle (*f.s.* 1) connected with its spiracular branch (*sp. br.*). The second, probably metathoracic, non-functional spiracle (*n.s.* 2) reduced to an external scar connected with an obliterated tube forming a scar filament (*sc.f.* 1) arising from an internal scar-like structure (*i.sc.* 1). The third non-functional first abdominal spiracle (*n.s.* 3) is also connected by means of a scar filament (*sc.f.* 2) with the internal scar (*i.sc.* 2) of the tracheae. Figs. 4–6, *N. del.* (Compare with Pl. III, fig. 1; Pl. VII, fig. 15; Pl. X, fig. 23; and Pl. XVIII, fig. 48.)

PLATE VI.

Pediculus humanus.

Fig. 7. Terminal portion of first left leg of the female.

Fig. 8. Terminal portion of the first left leg of the male. Note the highly developed thumb, and larger size of whole part compared to that of the female.

Fig. 9. Terminal portion of the third leg of female.

Fig. 10. Serrate plate, frontal aspect (*s.p.* Fig. 9).

Fig. 11. Terminal portion of first leg of first stage larva.

Letters common to Figs. 7–11, *N. del.*: *cl.* claw; *gr.* groove inside of which the serrate plate moves; *lm.* lamella; *m.l.* ligament of the flexor muscle of claw; *s.h.* sensory hair, two of many left unlettered in figures; *spn.* chitinous spine of the thumb; *tb.* tibia; *th.* thumb; *ts.* tarsus.

Plate VII.

Pediculus humanus.

Fig. 12. Lateral view of a male (*capitis*) heavily chitinised and pigmented, showing the pleural plates and transverse dorsal bands.

Fig. 13. Dorsal view of the head of a darkly pigmented specimen (*corporis*).

Fig. 14. Ventral view of same.

Fig. 15. Right portion of the thorax of this specimen seen dorsally. *ch.r.* chitinous ribs; *p.* thoracic pit; *sp.* functional spiracle.

Fig. 16. Left first leg of the third stage larva of male undergoing a moult (compare with Pl. VI, figs. 7, 8, and 9). Note position of old tendon when being thrown off from newly forming leg. (Figs. 12–16, *N. del.*)

Plate VIII.

Pediculus humanus.

Fig. 17. Portion of the femur of the first stage larva showing by transparency a polyscolopal chordotonal organ. *cu.* cuticle; *ch.o.* polyscolopal chordotonal organ; *g.ch.o.* nerve ganglia of chordotonal organ; *hy.* hypodermis; *m.* muscles; *n.* nerve branch to chordotonal organ; *sc.* scolopal organ; *s.g.* nerve ganglion of sensory hair—*s.h.*; *t.l.* terminal ligament of chordotonal organ.

Fig. 18. Third leg of the first stage larva seen by transparency. *cl.* claw; *ch.o.* monoscolopal chordotonal organ; *fm.* femur; *m.fl.cl.* muscles, flexors of claw; *m.fl.tb.* muscles, flexors of tibia; *m.ex.tb.* muscles, extensors of tibia; *n.* main nerve branch of the leg; *s.g.* nerve ganglion of sensory hair; *tb.* tibia; *ts.* tarsus. (Figs. 17, 18, *K. del.*)

Plate IX.

Pediculus humanus.

Fig. 19. Antenna of the first stage larva examined *in vivo* and showing by transparency the following structures: *cho.* 1 and *cho.* 2, two biscolopal and *cho.* 3, one monoscolopal chordotonal organ; *M.* muscles of basal segment of antenna; *N.* antennal nerve divided into two branches—*N* 1 and *N* 2; *s.g.* sensory ganglia which are at the base of about 15 sensory hairs—*s.h.*; *s.p.* sensory papillae with their ganglia (*s.g.* 1) and sensory tuft of 4 hairs with tube penetrating into sensory ganglion, *s.g.* 2; *s.t.* sensory tuft.

Fig. 20. Sensory organs on the ventral side of trochanter (*tr.*) of the second leg of second stage larva. *N.* nerve branch; *s.g.* sensory ganglion; *s.h.* sensory hair; *s.p.* sensory pits with *s.t.* sensory tubes forming a new type of sensory organ; *cox.* coxa.

Fig. 21. Sensory organs of the head. *ch.* chitinous layer of the head behind the eyes; *hy.* hypodermis; *s.h.* sensory hairs; *s.r.* sensory rods of chitinous structure, originating from the base of sensory hairs and penetrating into nerve ganglia which are not shown in this section.

Fig. 22. Posterior portion of the abdomen of the first stage larva seen dorsally and showing by transparency: *ao.* aorta; *al.m.* alary muscles; *ht.* heart; *n.* two nerve branches supplying the heart muscles; *os.* ostioles; *tr.t.* tracheal trunk. (Figs. 19–22, *K. del.*)

PLATE X.

Pediculus humanus.

Fig. 23. First stage larva seen dorsally showing by transparency the tracheal system (*N. del.*): *a.c.* 1, *a.c.* 2, and *a.c.* 3, three transverse anterior commissures with their secondary branches which ramify inside the nerve ganglia of the ventral chain; *f.s.* 1, the first pair of functional spiracles, probably the prothoracic; *f.s.* 4–9, the six functional abdominal spiracles; *h.c.* transverse head commissure; *i.sc.* 2, internal scars of the second non-functional spiracular branch (probably metathoracic); *i.sc.* 3, internal scars corresponding to the third non-functional pair of spiracular branches (probably first abdominal); *p.c.* posterior transverse commissure; *r.p.* tracheoles of rectal papillae; *sp.br.* spiracular tracheal branch; *tr.t.* main longitudinal tracheal trunk; *v.d.* ventral disc of the mid-gut, a structure containing the filaments of a fungus transmitted hereditarily from one generation of *Pediculus* to another (see Sikora, 1916). (Compare Fig. 23 with Pl. V, fig. 6 and Pl. XVIII, fig. 48.)

PLATE XI.

Pediculus humanus.

Fig. 24. Male. *N. and K. del.* Dissection showing the internal organs. The figure is drawn from one dissection made in the following way: the starved insect, rapidly killed in water warmed to 80° C. was put in 30 per cent. alcohol. The skin was cut along the lateral line and the dorsal wall was carefully separated from the rest of the body without disturbing the relative positions of the internal organs. The preparation was then stained in a weak solution of haemalum and was cleaned from fat body and tracheae. The vesicula seminalis was slightly displaced towards the right side of the body to render the view of the other organs clearer. The dissection, which was drawn from the dorsal side, shows:

(*a*) *The alimentary organs* beginning with oesophagus (*Oes.*); the anterior diverticula (*Div.*) of the mid-gut which becomes narrower in the posterior half of the body, bends sharply forward, and in the fourth abdominal segment passes into the hind-gut which bends backward, forms a rectal ampulla containing the rectal papillae, and passes into the rectum. From the dorsal and ventral sides of the region separating mid-gut and hind-gut arise the two pairs of malpighian tubules (*Malp.*). The anterior portion of the dissection shows the two pairs of salivary glands, the oval anterior pair (*Sal.gl.* I.) and the tubular posterior pair (*Sal.gl.* II.) attached to the dorsal surface of the mid-gut. Each of the latter glands is composed of two curved branches.

(*b*) *Nervous system:* the ventral chain, condensed into three nerve ganglia (*Gangl.*), is shown beneath the oesophagus.

(*c*) *The heart* with ostioles and alary muscles is shown on the medio-dorsal line, occupying the posterior portion of the fifth, and the anterior portion of the sixth abdominal segments; its anterior portion communicates with the aorta which runs along the median line. The anterior portion of the aorta was slightly shifted towards the left side in order to show the oesophagus.

(*d*) *The male genital organs* consisting of two pairs of pear-shaped testes, each pair being apposed basally where they open into a common vas deferens. The vasa deferentia leading from the paired testes on each side, terminate in corresponding branches of the forked vesicula seminalis. The vesicula in turn leads to the ductus ejaculatorius (*Duct.ej.*). Other parts figured are the basal plate, vesica penis (*B.Pl.* etc.) and dilator (*Dil.*). (See Nuttall, H. 1917, regarding genitalia.)

Plate XII.

Pediculus humanus.

Fig. 25. Transverse section of the wall of the mid-gut.

Fig. 26. Section of the anterior diverticula of mid-gut during secretory activity; *ep.* epithelium; *n.* nuclei of the epithelium.

Fig. 27. Section of the salivary gland (*s.g.*) and the fat body cells (*f.c.*) surrounding it. (See *Sal.gl.I*, Pl. XI, fig. 24.)

Fig. 28. Longitudinal section of a portion of ovarian tube showing the follicular epithelium (*f.e.*), ramified nuclei (*n.*) of nutrient cells (*n.c.*), and young ovule (*ov.*) with its spherical nucleus. (See Pl. XIII, fig. 36; and Pl. XV, fig. 45.)

Fig. 29. Section of follicular epithelium showing various stages of division of nuclei and of the rod-shaped nucleoli. (See *f.e.* Fig. 28.)

Fig. 30. Transverse section of the lateral portion of an abdominal segment showing the dorso-ventral muscles (*d.v.m.*); fat body cells (*f.c.*) and oenocytes (*oen.*).

Fig. 31. Longitudinal section of malpighian tubule. *l.* lumen; *n.* nucleus of the cell. (Figs. 25–31, *K. del.*)

Plate XIII.

Pediculus humanus.

Fig. 32. Transverse section of the head showing: *br.* brain; *f.c.* fat body cells; *l.* lens of eye; *o.g.* optic ganglion; *o.n.* optic nerve; *r.l.* retinal layer; *ph.* pharynx; *s.pr.* sac of piercing apparatus.

Fig. 33. Transverse section of the head showing: *cu.* cuticle; *f.c.* fat body cells; *hy.* hypodermis; *ph.* pharynx; *s.g.h.* salivary glands of the head; *sph.* sphinctor muscles; *s.pr.* sac of the piercing apparatus.

Fig. 34. A portion of distended accessory gland of the genital organ of a female containing an egg in uterus. (See Pl. XVI, fig. 46.) *ep.* epithelium of the gland; *m.* ramified system of muscles.

Fig. 35. The receptaculum seminis with its ramified muscular system (*m.*). It arises from the anterior portion of the uterus (*u.*).

Fig. 36. The five ovariole tubules, two partly overlapping, seen in optical section, showing: *f.e.* follicular epithelium; *gm.* germinal cells or germarium; *n.c.* nutritive cells; *od.* oviduct; *ov.* oocytes in various stages of development; *t.f.* terminal filaments.

Fig. 37. Binucleate nephrocyte cell, slightly compressed: *e.i.* excretory inclusion; *n.* the nuclei. (Figs. 32–37, *K. del.*)

PLATE XIV.

Pediculus humanus.

Fig. 38. A portion of the abdominal fat body of an adult *Pediculus* showing nuclei with scattered chromatic granules.

Fig. 39. Head of first stage larva showing by transparency the fat body cells (*f.c.*) containing the masses of excretory granules (*e.g.*), probably urates. (See Pl. IV, fig. 3.)

Fig. 40. Blood cells or cells of perivisceral fluid, some of which contain vacuoles with phagocytised inclusions.

Fig. 41. Longitudinal section of an abdominal spiracle showing: *at.* spiracular atrium; *cu.* cuticle of the body; *hy.* hypodermis; *m.cl.* muscles of the closing apparatus; *sp.f.* spiracular felt chamber; *sp.o.* spiracular opening.

Fig. 42. Section of the body wall near the posterior end of the female showing: *e.cu.* external cuticle; *hy.* hypodermis; *i.cu.* internal cuticle; *n.* nerve; *n.g.* nerve ganglion of the sensory hair (*s.h.*); *s.r.* sensory rod. (See *sg.* 2, Pl. IX, fig. 19, and *s.r.*, Pl. IX, fig. 21.)

Fig. 43. A portion of a section of egg operculum showing the air chamber (*a.ch.*) and the internal pore (*in.p.*) leading to a series of funnel-shaped spaces representing the internal micropyle of the egg.

Fig. 44. Section of operculum. *a.ch.* air chamber; *ex.p.* external pore; *in.p.* internal pore; *op.s.* opercular suture; *w.* wall of air chamber. (Figs. 38–44, *K. del.*)

PLATE XV.

Pediculus humanus.

Fig. 45. Female genital organs showing: *a.g.* accessory glands arising from uterus; *ap.* chitinous apophyses for muscular insertion; *gn.* gonopods; *od.* oviduct; *ov.* ovum; *o.vl.* five pairs of ovariole tubes forming the ovary (*ovr.*); *r.s.* receptaculum seminis; *ut.* contracted uterus; *vg.a.* anterior portion of vagina; *vg.p.* posterior portion of vagina; *v.m.* vaginal muscles; *sp.* spiracle. *K. del.*

Plate XVI.

Pediculus humanus.

Fig. 46. Female genital organs showing the egg enclosed in a distended uterus. *a.g.* accessory glands; *ex.p.* external pore of micropyle of the operculum (*op.*) of the egg (*ov.*) lying inside and distending the uterus (*ut.*); *r.s.* receptaculum seminis overlying the operculum with the micropyle pores; *vg.* vagina. *K. del.* (See Pl. XV, fig. 45.)

Plate XVII.

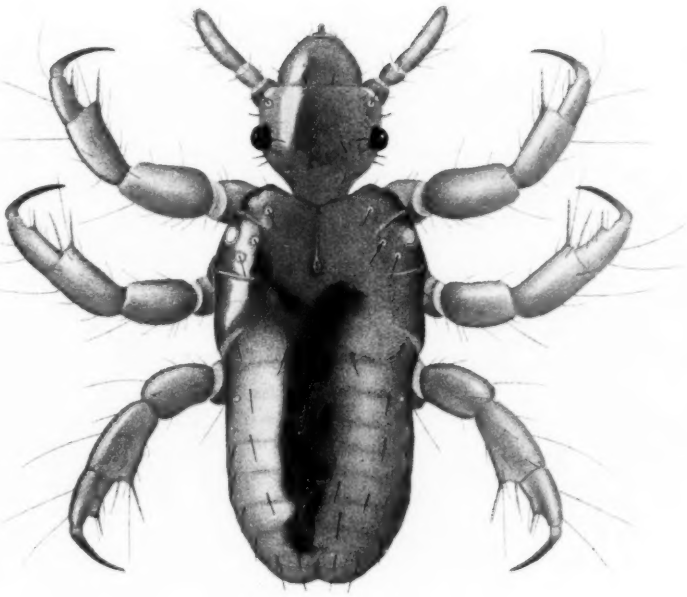
Pediculus humanus.

Fig. 47. Frontal section of the posterior end of the body of a female, showing: *f.b.* fat body; *m.t.* sections of malpighian tubules; *ov.* ovum lying within the uterus; *vg.* vagina showing the denticulated surface of the cuticle; *ut.* uterus; *v.m.* vaginal muscles. *K. del.*

Plate XVIII.

Phthirus pubis.

Fig. 48. Adult female seen dorsally, showing by transparency the tracheal system: *a.c.* 1, *a.c.* 2, and *a.c.* 3, three transverse anterior commissures with their secondary branches which ramify within the nerve ganglia of the ventral chain; *f.s.* 1, the pair of functional spiracles, probably the prothoracic; *f.s.* 4–9, the six functional abdominal spiracles; *h.c.* transverse head commissure; *i.sc.* 2, internal scar of the second non-functional spiracular branch (metathoracic); *i.sc.* 3, internal scar of the third pair of non-functional spiracular branches (probably first abdominal); *n.s.* 2, non-functional spiracle (second or metathoracic); *n.s.* 3, non-functional third spiracle (probably first abdominal); *p.c.* transverse posterior commissure; *r.p.* tracheoles of rectal papillae; *sc.f.* scar filaments, or obliterated spiracular branches of the second and third pairs of non-functional spiracles; *tr.t.* the main longitudinal tracheal trunks; *v.d.* ventral disc of the mid-gut. *N. del.* (Compare with Pl. V, fig. 6; and Pl. X, fig. 23.)



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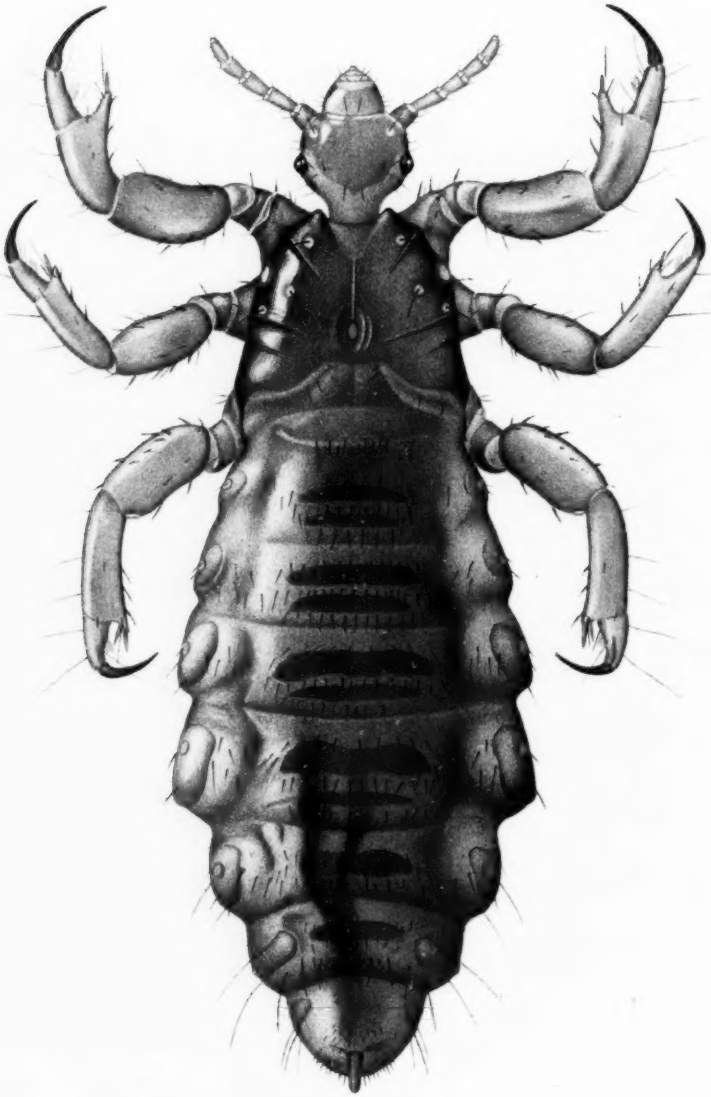

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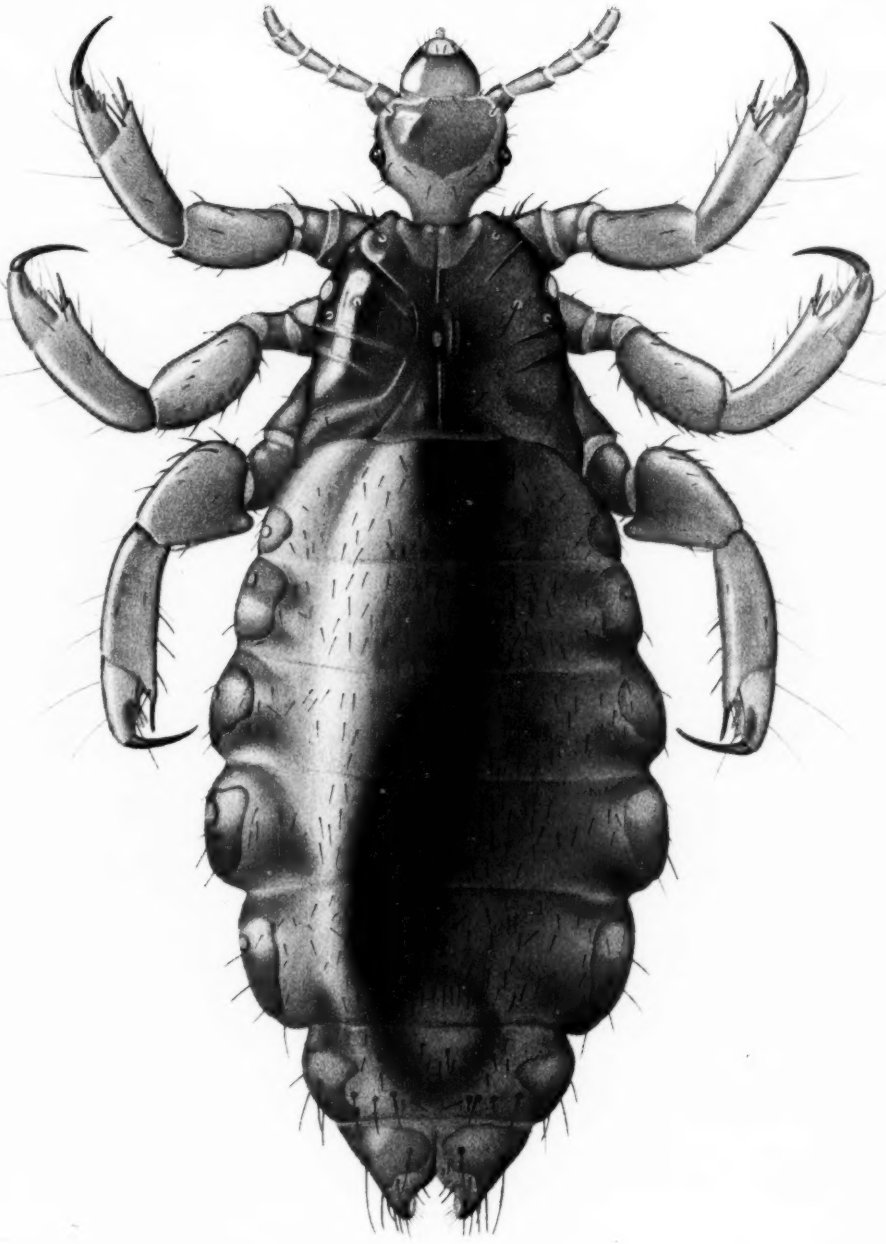

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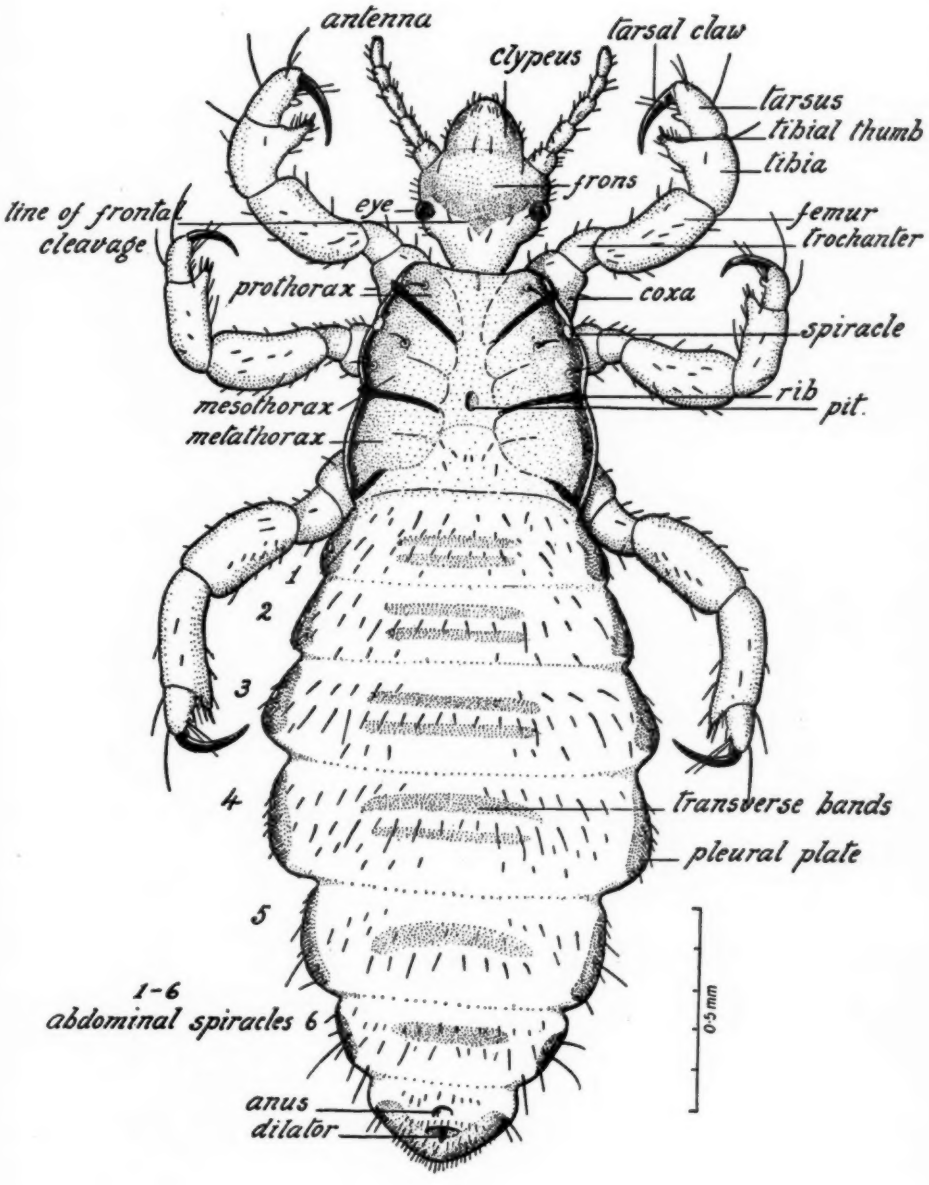
antenna
clypeus
tarsal claw
tarsus
tibial thumb
tibia
frons
eye
line of frontal cleavage
femur
trochanter
prothorax
coxa
spiracle
rib
pit
mesothorax
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pleural plate
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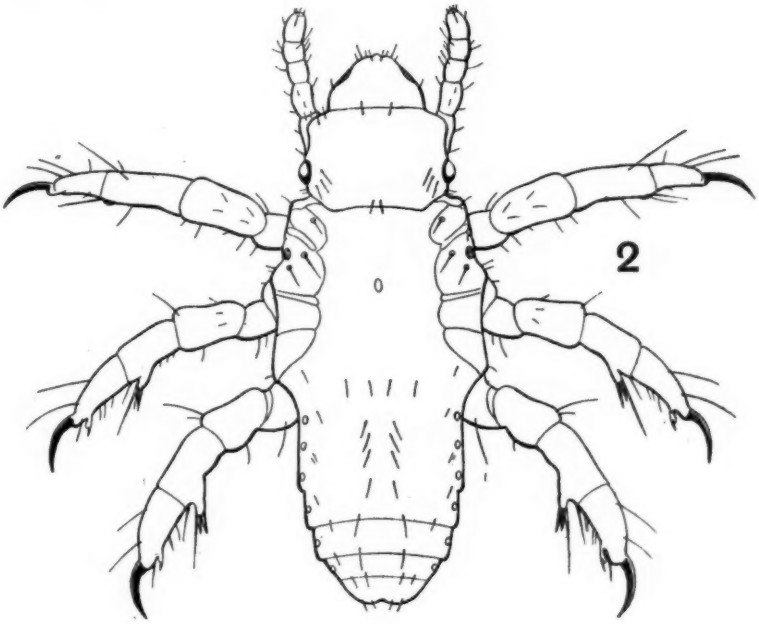
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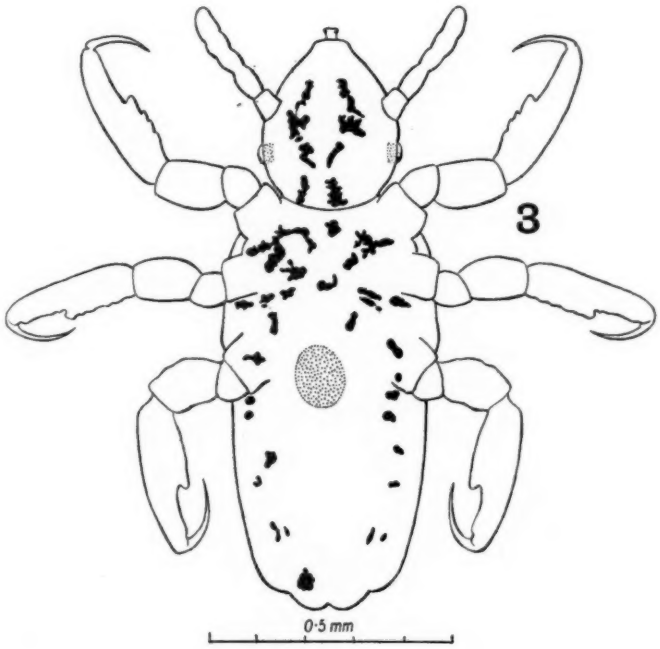
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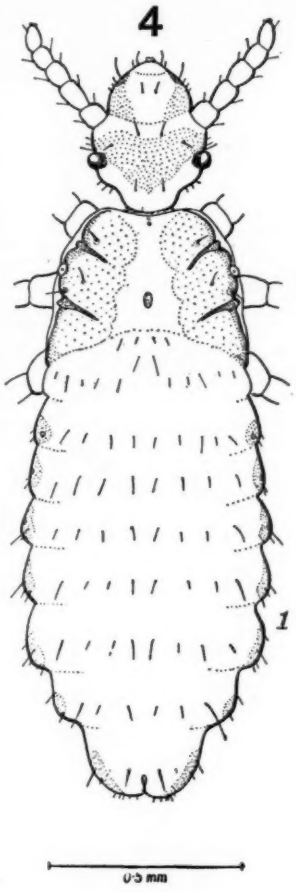
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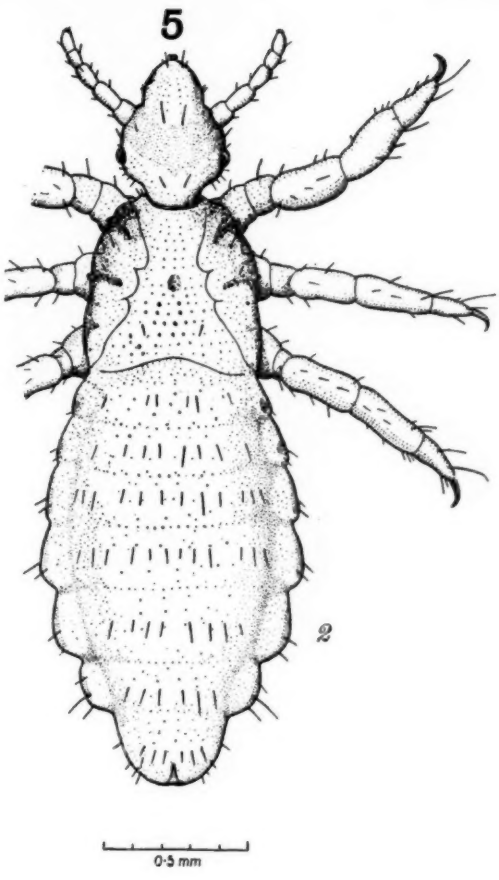
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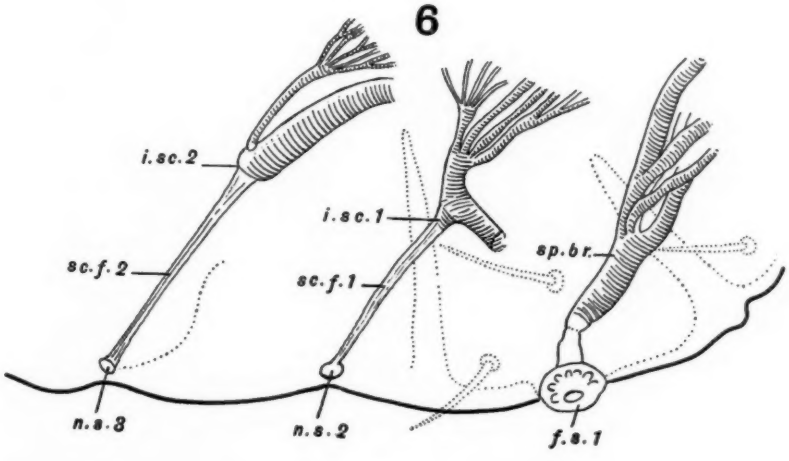
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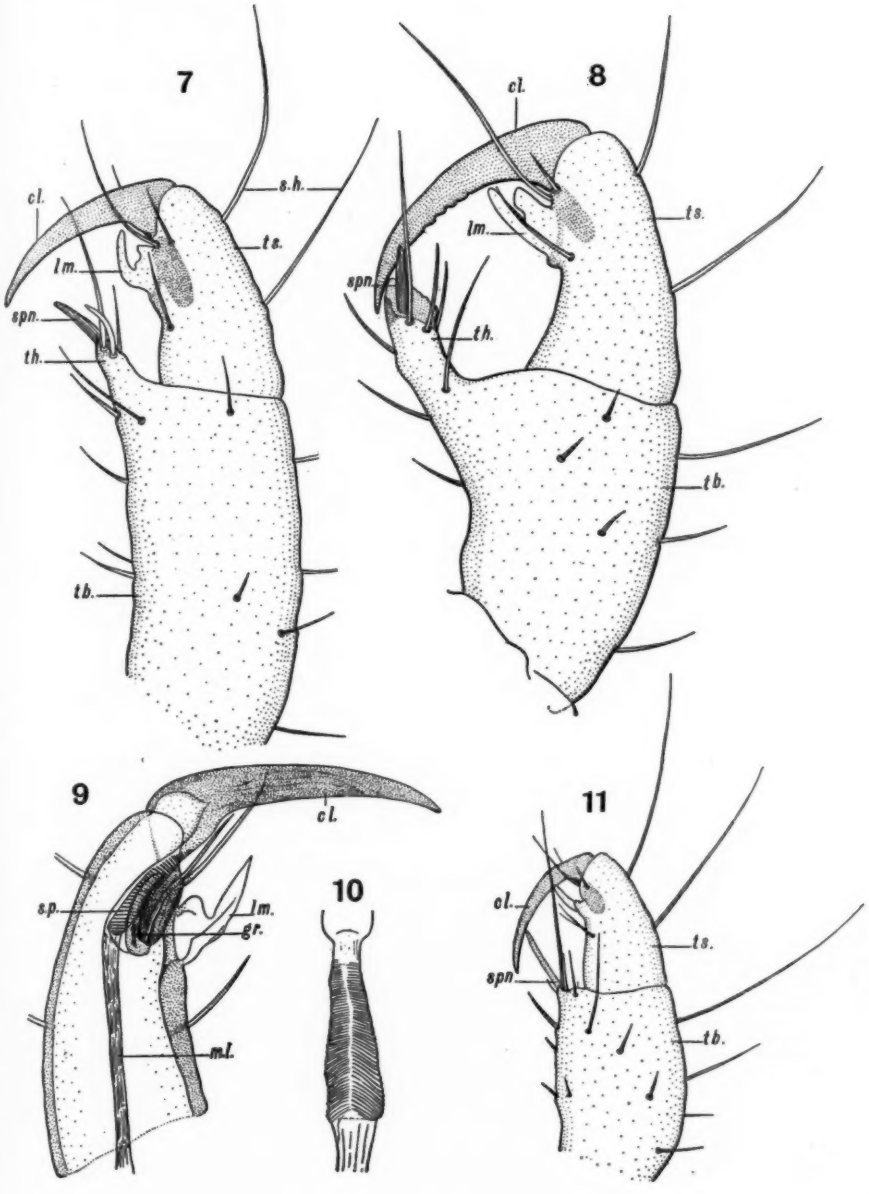
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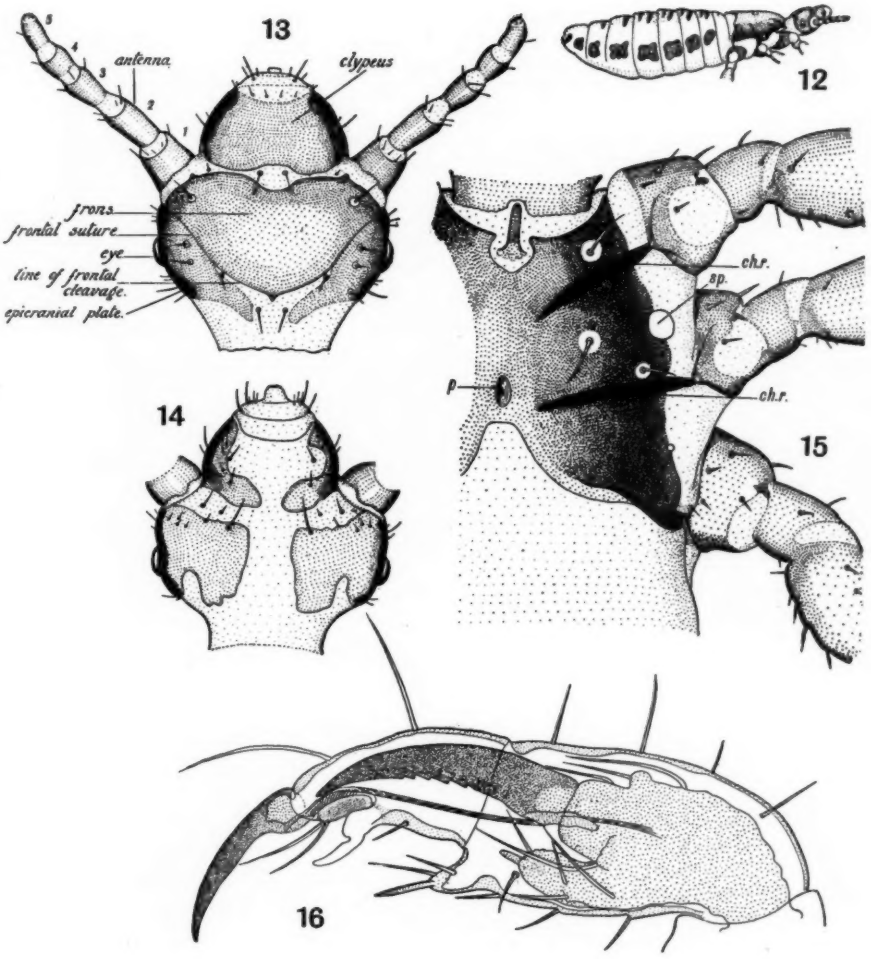
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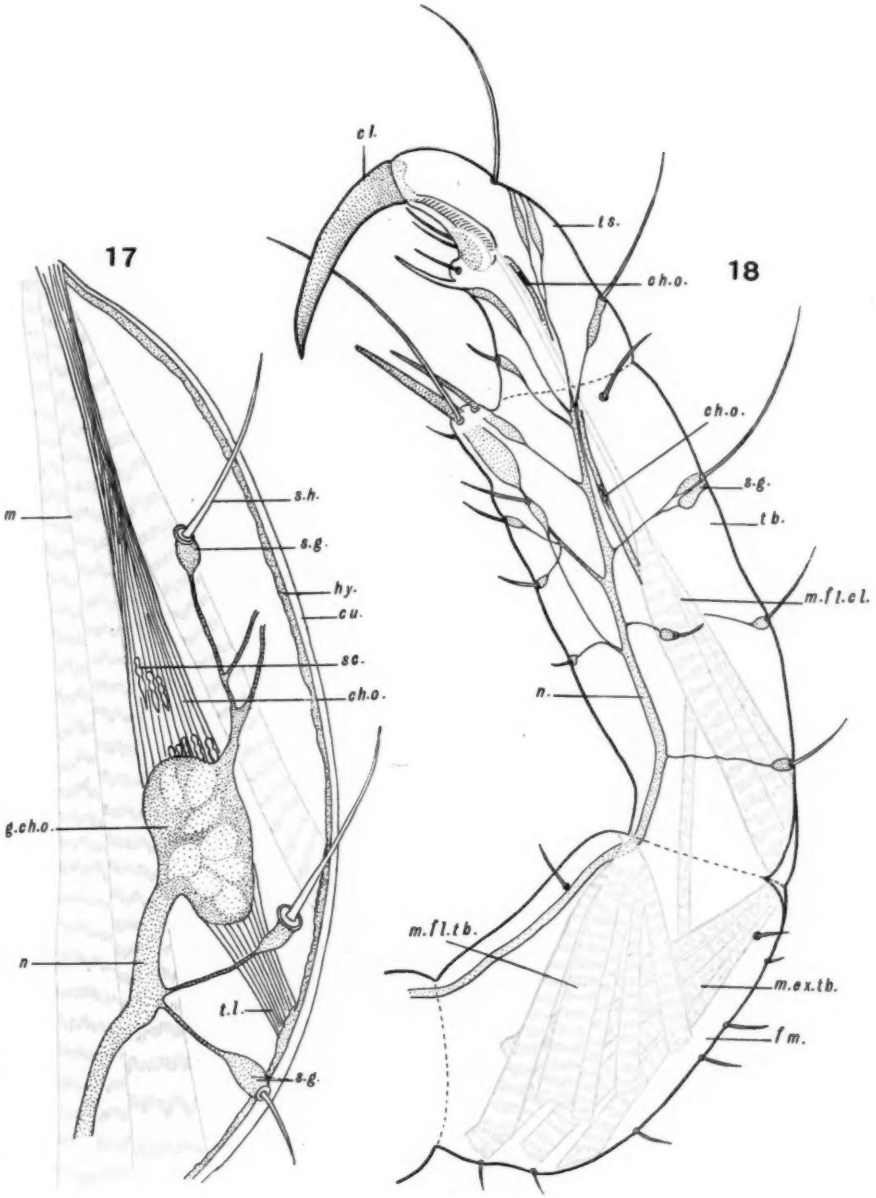
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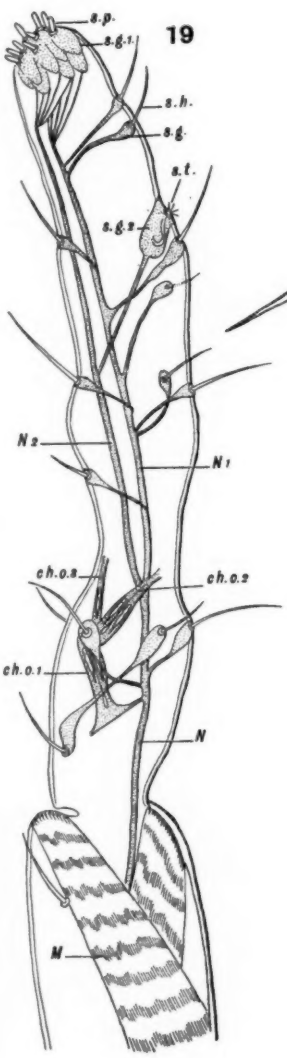
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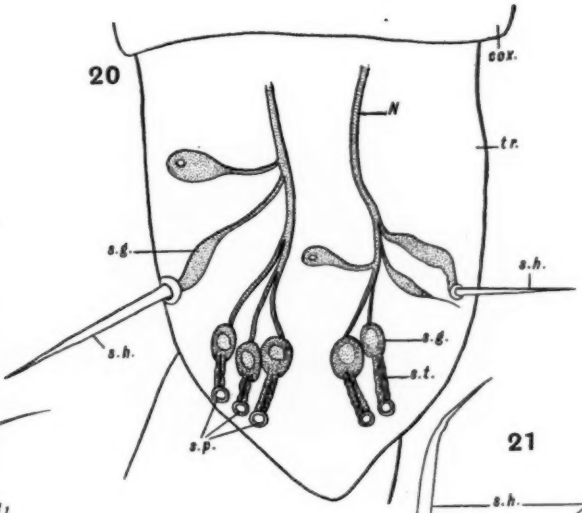
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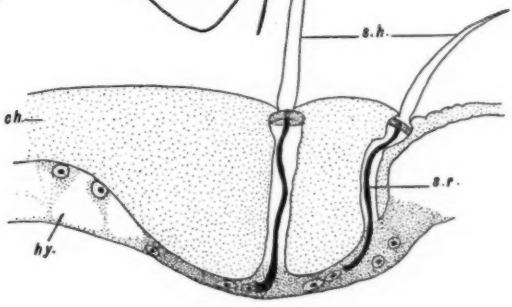
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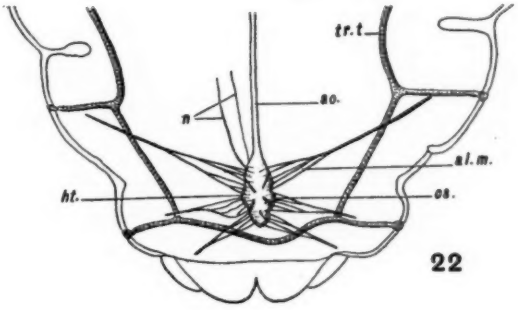
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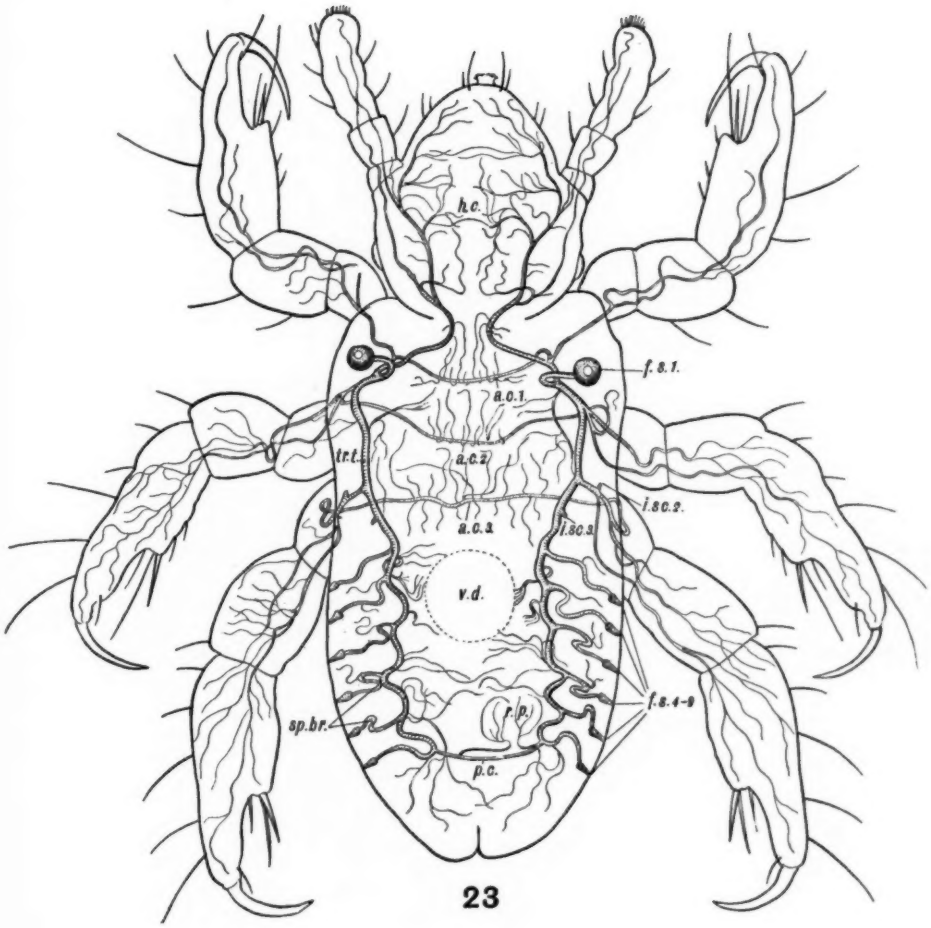

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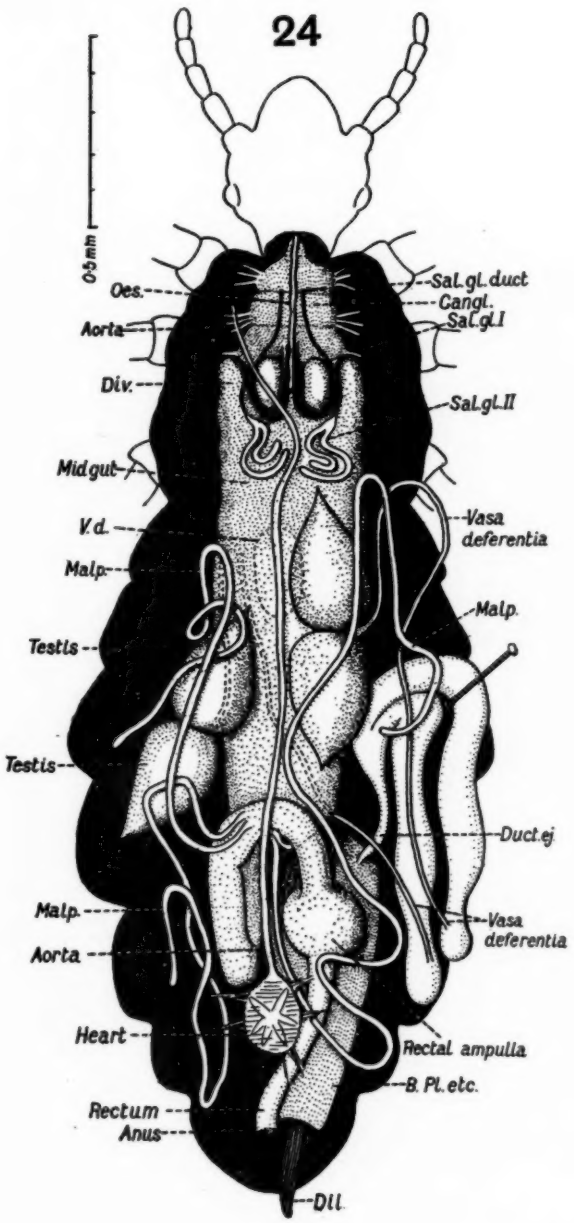
24
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Testis
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Heart
Rectum
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Dil.
Sal.gl.duct
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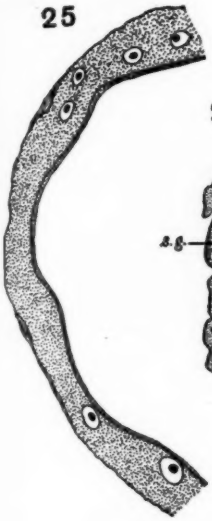
25



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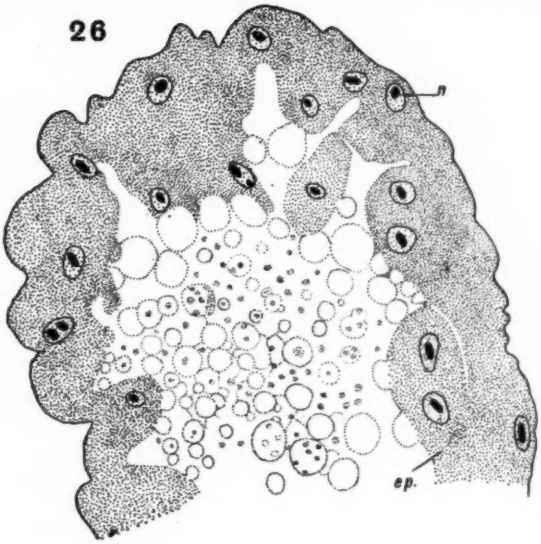

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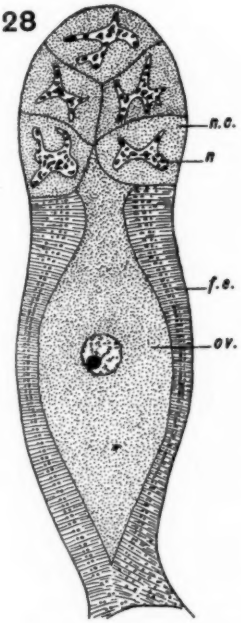

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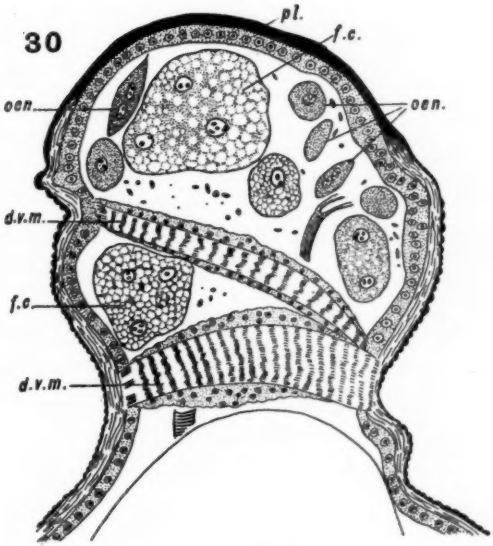

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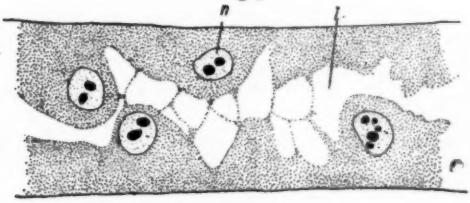

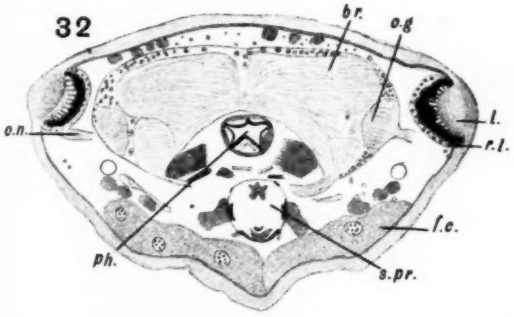
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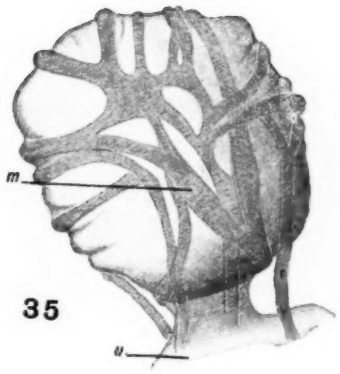
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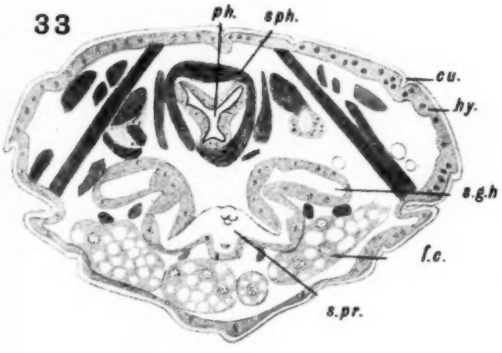
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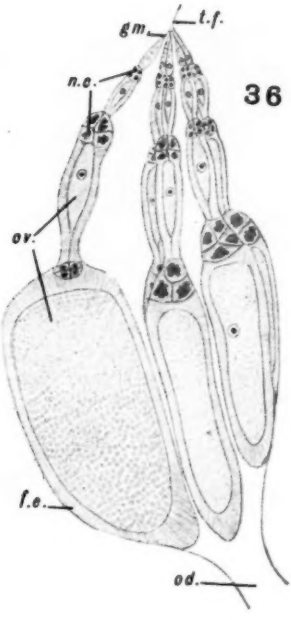
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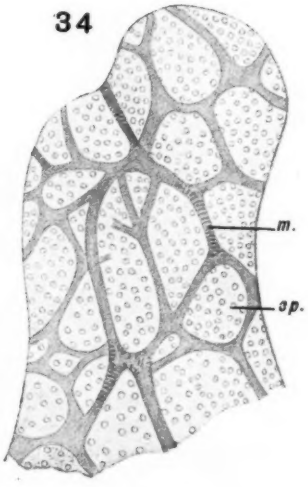
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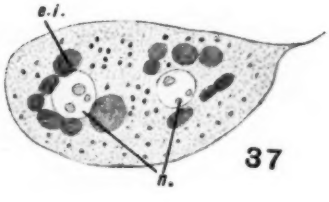
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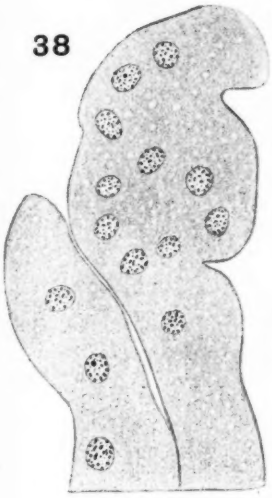

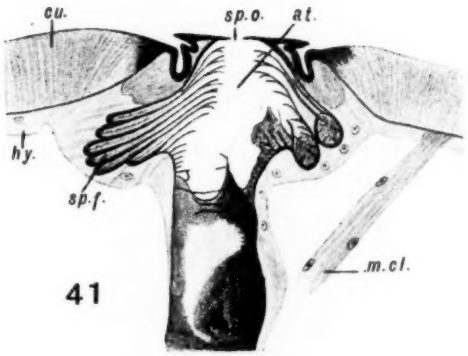
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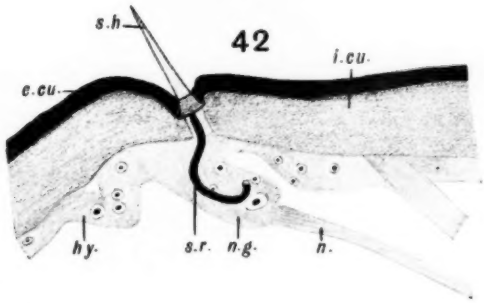
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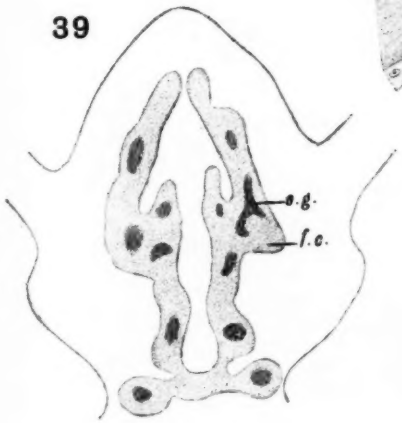
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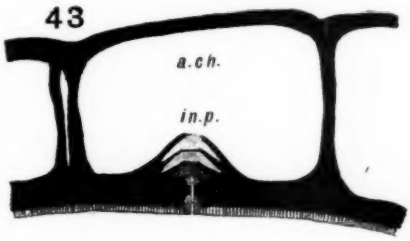
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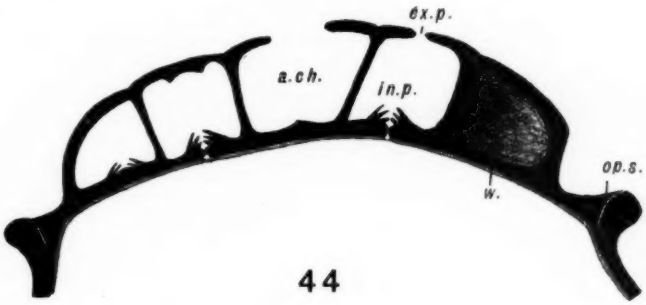
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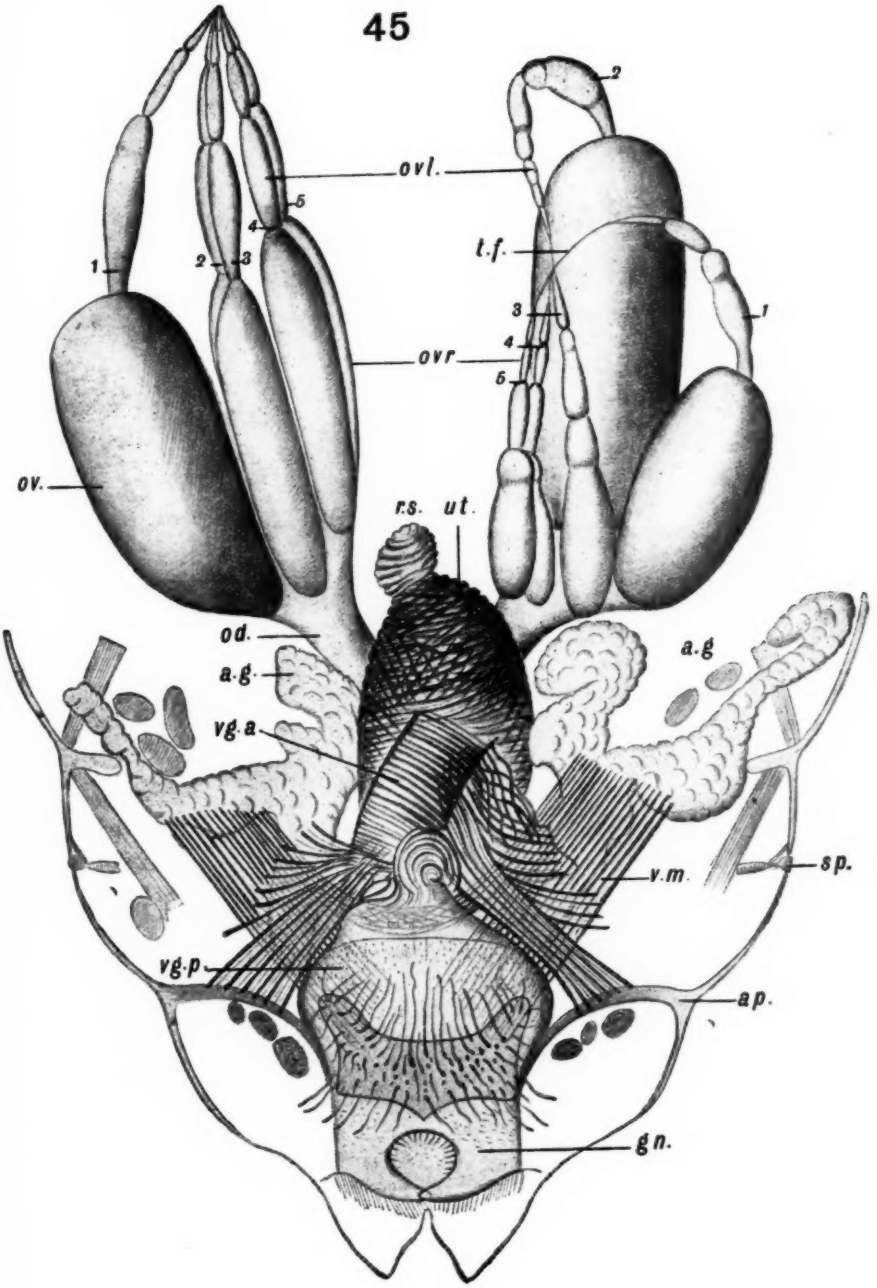
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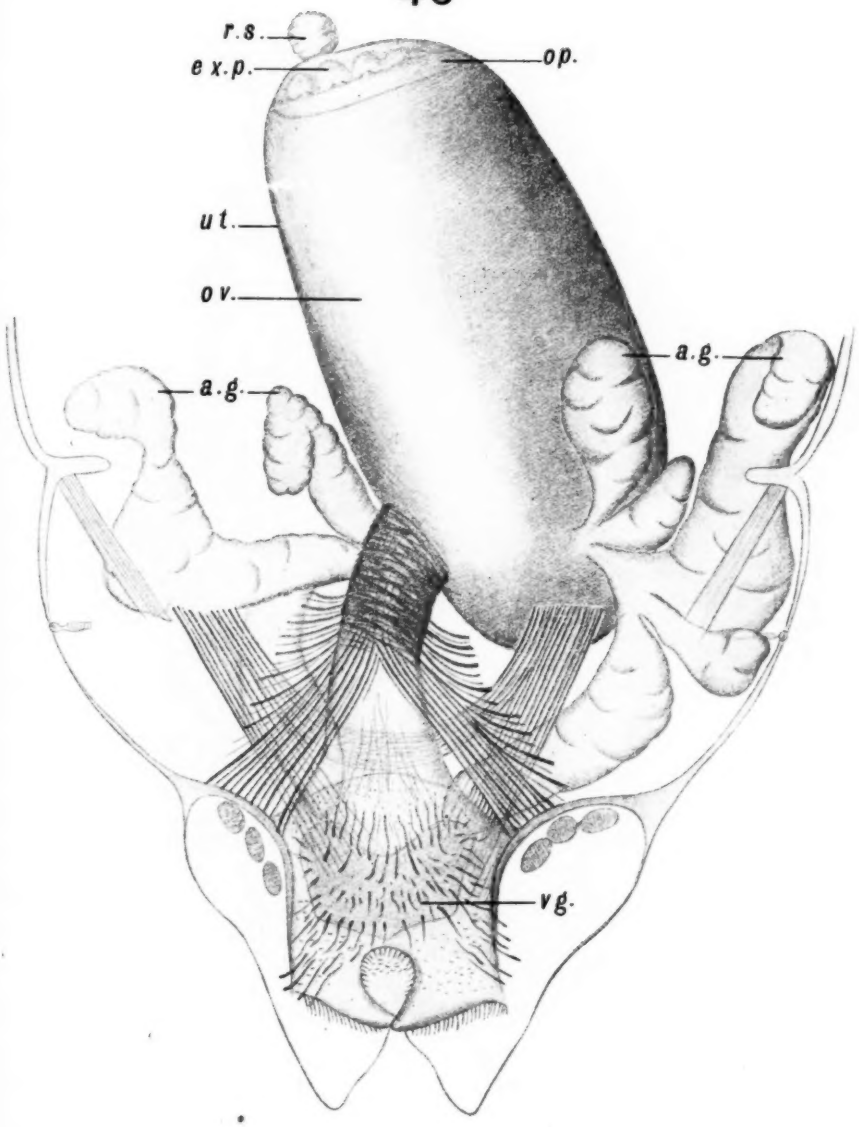

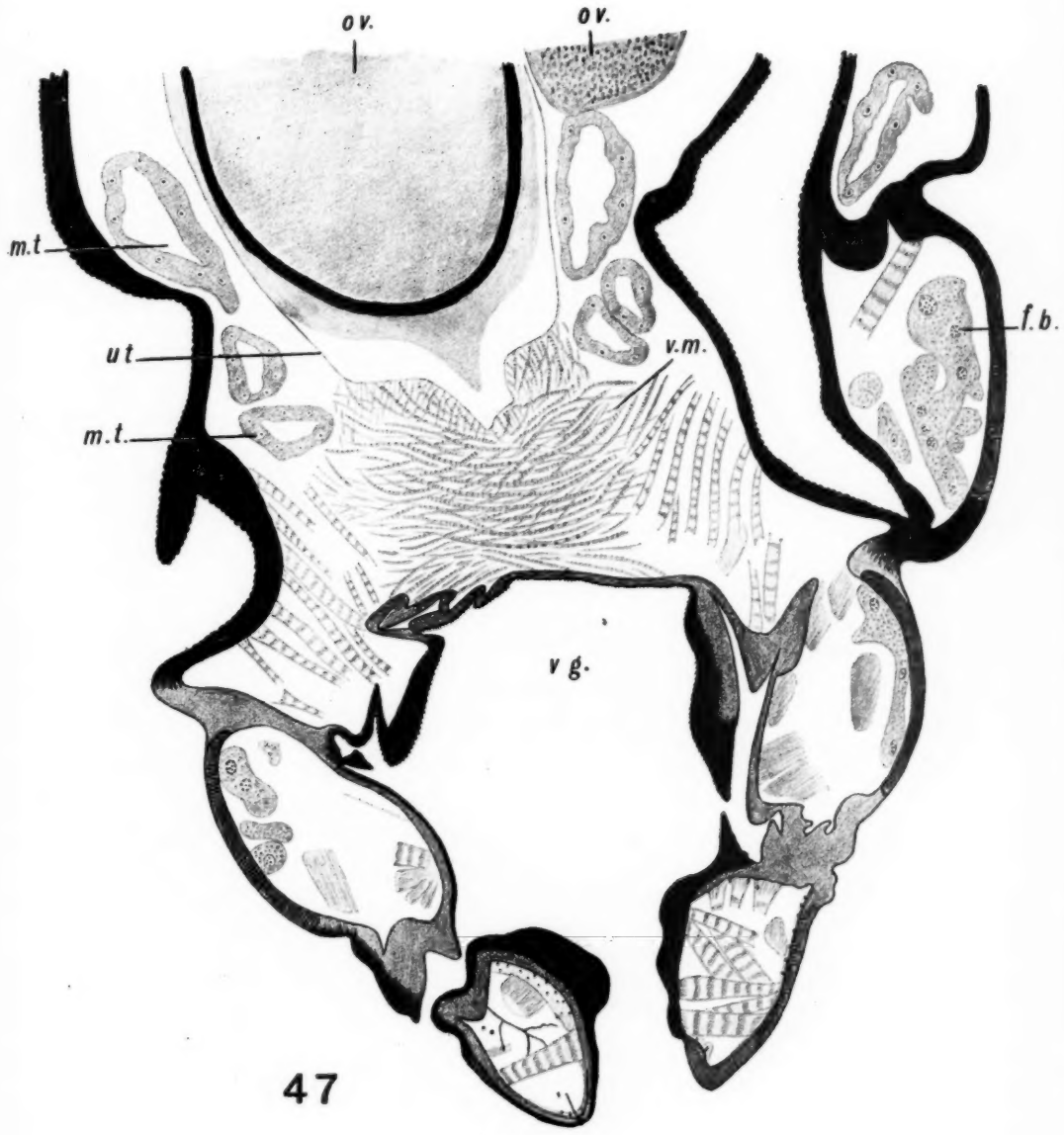

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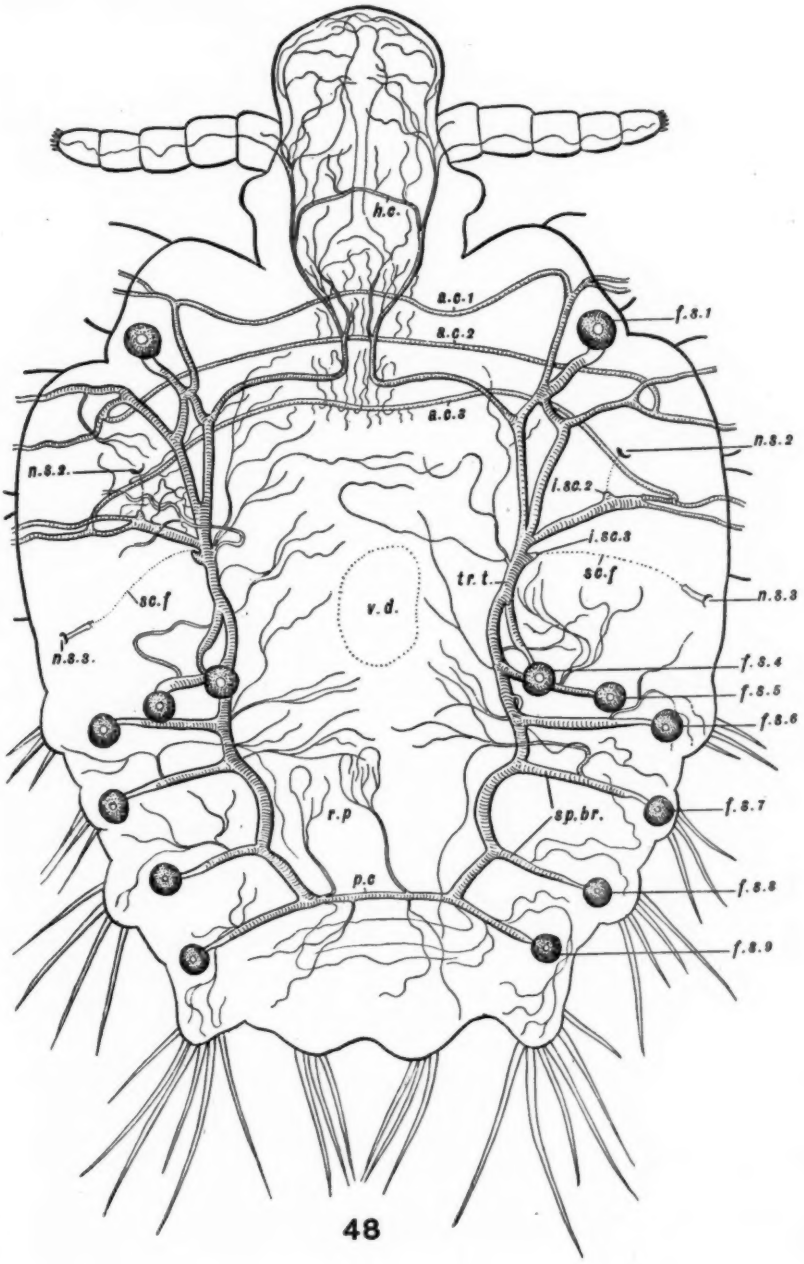
PARA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f.s.8
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—— (XI. 1917). The part played by *Pediculus humanus* in the causation of disease. *Ibid.* **10**, 43–79, Pl. I.

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NOTE. The last three bibliographies cover a wider field than the first since they include Pediculidae in general and Mallophaga. The last contains a number of titles of more recent date together with some omitted in the earlier bibliographies.

OBSERVATIONS ON SOME PARASITES OF *OSCINELLA FRIT* LINN.

PART I.

By A. D. IMMS, D.Sc., F.R.S.

Rothamsted Experimental Station, Harpenden.

(With 14 Text-figures.)

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1. Introductory remarks.

The frit-fly (*Oscinella frit* Linn.) is so well known as a pest of oats and also of other cereal crops that a detailed account of its biology is unnecessary. In a few words it may be said that, under English conditions, the flies of the first generation oviposit in April and May on the leaves or stems of spring oats and on various grasses. The larvae bore into the shoots causing the death of the central leaves and growing point. Flies of the second generation oviposit during July on the ears of oats and the larvae feed upon the spikelets and young grain. Oviposition by the third generation of flies occurs during August and September or later, the eggs being laid on the leaves of winter cereals and various grasses. Winter is passed in the larval condition at the bases of the shoots which are ultimately destroyed and the over-wintering larvae give rise to the first generation of flies of the next year.

Frit-fly is especially destructive in the southern half of England, but also occurs as far north as Northumberland and has been recorded as doing damage in Scotland and Ireland. It is prevalent in most countries of continental Europe and is very widely distributed in North America.

The object of the present studies is to obtain information as to the extent to which frit-fly is destroyed by hymenopterous parasites, the species of the latter that are present and their economic significance. In carrying out these observations valuable assistance has been rendered by Dr J. Waterston of the British Museum in the identification of the parasites bred out and also by Dr H. Hedicke of Berlin with reference to the same matter.

2. Methods of investigation.

In order to ensure an adequate supply of material for investigation, oats (variety "Supreme") were sown in 1926 and 1927 in plots set apart for the purpose on the Rothamsted farm. The plots were parts of fields already sown with oats and were consequently favourably situated with respect to becoming infested with frit-fly. Both the years 1926 and 1927 proved to be exceptional on account of the relatively slight infestation of frit-fly, and this same feature was recorded by the Ministry of Agriculture (1928) from most other parts of the country. In the Harpenden district the comparative scarcity of the insect hampered the investigation in that it greatly increased the time and labour involved in collecting material for study. The amount of material ultimately obtained was markedly less than it was anticipated would be available. During 1926 the oats were sown on a single plot on March 19th, and collections of infested plants were made at frequent intervals between June 29th and July 8th. Altogether 219 examples of frit-fly were bred and 84 parasites, the percentage of parasites being 27·7.

During 1927 three plots were used and details respecting these are given below.

Plot I. Date of sowing, March 15th; date of collection of pupae, June 27th; frit-flies bred, 138; parasites bred, 26; percentage of parasites, 15·8.

Plot II. Date of sowing, March 26th; date of collection of pupae, June 6th–7th; frit-flies bred, 128; parasites bred, 86; percentage of parasites, 40·1.

Plot III. Date of sowing, April 25th; date of collection of pupae, July 12th–13th; frit-flies bred, 72; parasites bred, 86; percentage of parasites, 54·4. Taken collectively 338 frit-flies and 198 parasites were bred from the three plots during 1927, the percentage of parasitism being 36·9.

On the dates indicated oat plants showing signs of infestation by frit-fly were pulled up and removed to the laboratory for examination. Each plant was then gone over and any larvae or puparia of frit-fly were removed. The puparia were carefully scrutinised and any which did not appear to be referable to the species in question were discarded. The remaining puparia were then transferred to cages containing a layer of steam-sterilised sphagnum moss. The cages were placed in an outdoor insectary, where the prevailing temperature varied only slightly from that of the open air. The cages were of an ordinary type used in rearing parasites and were little more than dark boxes with the front wall perforated for the reception of glass phials. Owing to their positive reaction to daylight, both hosts and parasites as they emerged congregated in the phials, where they were easily collected for examination.

Larvae dissected out of the stems were placed on discs of moistened filter paper and transferred to small glass receptacles and a number of them succeeded in completing their transformations. The object of keeping such larvae under observation was to ascertain whether the parasites attack their hosts while the latter are in the larval or pupal stages. From among 64 frit-fly

larvae retained in this way, 33 died, 17 produced flies and 14 produced parasites.

The above methods, although laborious, were adopted in order to make quite certain that the parasites bred were, in every case, species actually affecting *Oscinella frit*. In this connection it is noteworthy that all the flies that emerged from unparasitised pupae pertained to this species.

During 1926–7 attention was mainly confined to the parasites of the first or stem infestation of frit-fly: those of the second or grain infestation are not dealt with in the present article.

3. PARASITES REARED FROM *OSCINELLA FRIT*.

Six species of parasitic Hymenoptera were reared in the Harpenden district from puparia of *Oscinella frit* during the years 1926–7. Three of these species, viz. two Chalcids and a Braconid, were present only very rarely and an account of them is postponed until further material is available. The remaining three species, which are dealt with in some detail, are *Halticoptera fuscicornis* Walk. (Chalcidoidea), *Rhoptromeris eucera* Hartig (Cynipoidea) and *Loxotropa tritoma* Thoms. (Proctotrypoidea). Little is known concerning any of these three species and no detailed descriptions of them exist.

I. **Halticoptera fuscicornis** (Walk.).

The genus *Halticoptera* Spinola is placed in the family Miscogasteridae, a group which is doubtfully distinct from the Pteromalidae and only separable from the latter on comparatively trivial characters. *Halticoptera* occurs over the whole of Europe, Northern Africa and extends its range into both North and South America. It is closely allied to *Dicyclus* Walk. and both genera are characterised by the parapsidal furrows being incomplete and only evident anteriorly: also, in both genera the clypeus is transverse with a deep median sinus in its anterior margin. In *Halticoptera* the marginal vein is longer than the stigmal, whereas in *Dicyclus* it is usually shorter than or, at most, equal in length to the stigmal vein. The most striking difference, however, between the two genera is afforded by the maxillary palpi in the males. In *Halticoptera* the two distal joints are greatly inflated so that they form together a spheroidal vesicle, while in *Dicyclus* they are unmodified as in the females of both genera.

Published observations on the biology of *Halticoptera* are very scanty, but they indicate that it is a parasite of cyclorrhaphous Diptera and the following records are noteworthy. *H. smaragdina* (Curt.) is recorded by Curtis (1860) and by Billups (1883) from pupae of the celery-fly (*Acidia heraclei* L.) in England, but it has been doubted whether it is a primary parasite of the *Acidia* or a hyperparasite through the Braconid *Adelura apii*. Smith and Gardner (1922) recorded *H. flavicornis* Spin. as a common parasite of *Acidia heraclei* in England, a fact which lends support to the conclusion that *H. smaragdina* is a primary parasite also. *H. petiolata* Thoms. is recorded by Meyer (1923)

from *Oscinella frit* in Germany and also by Ruschka and Fulmek (1915) from Anthomyidae living in *Brassica oleracea* in Lower Austria. *H. suilius* Walk. is recorded by Schander and Meyer (1925) also from *Oscinella frit* in Germany, while *H. patellana* (Dalm.) is mentioned by Ruschka and Fulmek (1915) under the name *H. similis* Först. as being bred from puparia of Diptera parasitising *Agrotis tritici* L. in Lower Austria. Of especial interest is the species *H. daci* Silv. from Eritrea which has been introduced by Silvestri (1914) into Italy, along with other parasitic Hymenoptera, as an auxiliary biological agent in the control of the olive-fly *Dacus oleae* of which it is a

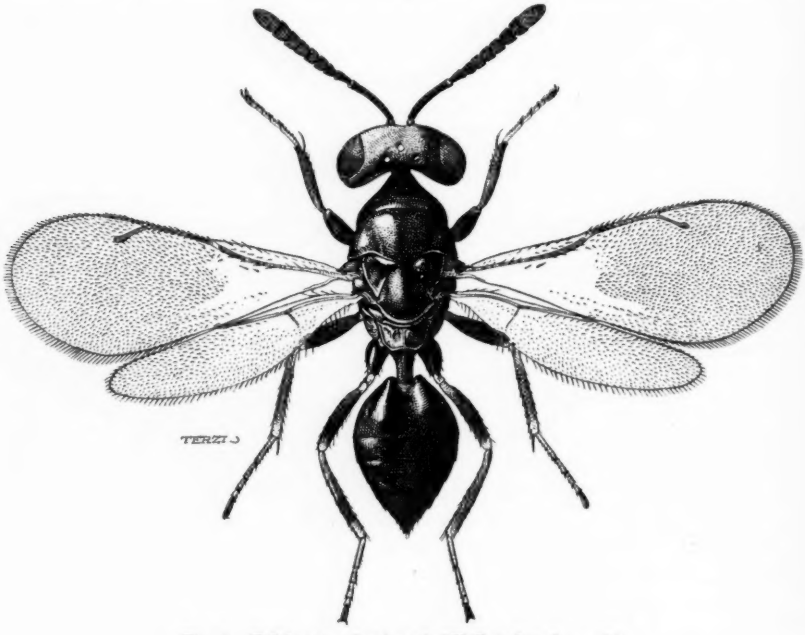

Fig. 1. *Halticoptera fuscicornis* (Walk.), female. × 36.

parasite. The species *H. brodei* Ashm. is described by Ashmead (1887) from the Cynipid *Biorhiza forticornis* (Walsh) in Canada, but it appears probable that it was parasitising a species of dipterous larva inhabiting the gall of the *Biorhiza*. Another doubtful record concerns the species *H.* (*Pachylarthrus*) *breviventris* Först. which is stated by Dours (1874) to have been bred by Goureau from the Coleopteron *Bruchus palladicornis* Schh.; the name *breviventris* it may be added is not recognised by Dalla Torre in his Catalogue (1898).

Halticoptera fuscicornis was described by Walker (1833) as *Dicyclus fuscicornis*, and the first record of it parasitising *Oscinella frit* is that of Cunliffe (1921) who reared it in 1919 from puparia of *O. frit* collected in the Oxford

district. Dr Cunliffe informs me that he has again reared it from the same host in 1926; it has also been bred by Mr T. H. Taylor from frit-fly puparia in the Leeds district. From an examination of collections of frit-fly parasites reared by Messrs Cunliffe and Taylor, it is evident that *H. fuscicornis* is common in the two districts mentioned. It is also prevalent around Harpenden (Herts.) and probably distributed through the greater part of England and Wales, but this point needs enquiry. Examples reared from host puparia collected at Harpenden emerged between July 7th and September 3rd, the majority appearing during the month of August. It is evident, therefore, that the species is on the wing during the whole of summer. It attacks its host as an endoparasite in the larval stage of the latter and finally completes its transformations within the puparia.

Description of the female (Fig. 1).

Coloration. Varying from dark metallic green to bronze-black, or bronze with the abdomen dark green; hairs whitish throughout. Eyes dark chocolate; ocelli translucent brown. Antennae with scape and pedicel dark metallic green or bronze; funiculus and club very dark fuscous or brown-black. Mandibles yellow-brown, teeth darker. Legs with coxae dark metallic green to bronze-black; trochanters smoky ochreous; femora dark bronzy brown with proximal and distal extremities ochreous; tibiae and tarsi ochreous often variably suffused with fuscous, in some examples all the tibiae are strongly infuscated; fifth tarsal joint and claws brown-black. Tegulae dark brown; wings hyaline with veins pale smoky yellow. Pedicel black; abdomen smooth and shining with green or bronze reflection.

Head. Facial aspect broader than long (Fig. 2), as 6 : 5, sparsely clothed with short hairs; occiput broadly and rather deeply concave. Eyes moderate sized, separated by a width slightly less than frontal length of head. Ocelli disposed as a broad-based triangle; lateral ocelli separated from orbits by their own distance apart. Toruli (*T* in Fig. 2) oval, separated by about their

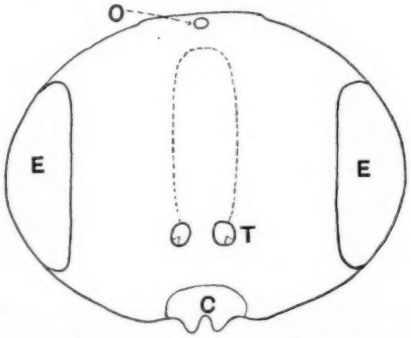

Fig. 2. *Halticoptera fuscicornis*, female: outline of frontal aspect of the head, ×circa 100. *C*, clypeus; *E*, compound eye; *O*, median ocellus; *T*, toruli.

own length and by nearly three times that length from a base line passing through the floor of the clypeal sinus. A rather broad, shallow median longitudinal furrow (Fig. 2) extends from behind the toruli to about three-fourths of the distance to the median ocellus. Clypeus (Figs. 2 and 3) with two projecting processes separated by a median sinus. Labrum (Fig. 3 D) sub-triangular with a median anterior process bearing a pair of greatly flattened, spatulate setae[1]. The whole surface of the head is sculptured with a reticulation of mostly hexagonal or pentagonal cells (Fig. 3 I).

Antennae (Fig. 3 A). Length 0·8 mm.; scape 0·23 mm. long × 0·03 mm. broad, related in length to pedicel as 7 : 2. Pedicel longer than any of the funicular joints which are sub-equal in length. The two ring-joints together slightly shorter than first funicular joint; club 3-jointed, related in length to last funicular joint as 7 : 2. Sensoria vary slightly in number in different examples; their approximate numbers from first funicular joint to the apex of the club are respectively 7 : 7 : 7 : 7 : 9 : 10 : 11 : 15 : 8.

Mouth-parts. Mandibles (Fig. 3 C) of the two sides closely alike; 0·2 mm. long × 0·12 mm. maximum breadth; each with four teeth. Maxillary palpi 4-jointed; joints related in length as 4 : 12 : 8 : 15. Labial palpi 3-jointed; joints related in length as 2 : 1 : 4. Both pairs of palpi terminated by a stiff seta, longer than third joint of maxillary palpus.

Thorax (Fig. 1). Tergal, pleural and sternal sclerites of pro- and mesothorax with a reticulate pattern very similar to that of the head; pubescence almost wanting. Mesonotum with parapsidial furrows incomplete posteriorly, faint when viewed dorsally but evident when seen laterally. Scutellum almost hemispherical in profile from side to side; posterior margin with a raised border. Metanotum band-like, its surface devoid of reticulation. Propodaeum (Fig. 3 H) with a median carina and a lateral carina on either side, the three carinae merging posteriorly into a transverse ridge; on each side, in front of the spiracle, is a group of rather long hairs which are continued down each side margin; posteriorly the propodaeum bears a median socket which receives the anterior end of the pedicel. Fore wings (Fig. 1) hyaline, rather more than twice as long as broad; length (four examples) 1·4 mm. to 1·8 mm., breadth 0·61 mm. to 0·82 mm. (including marginal hairs). Distal two-thirds of surface for the most part uniformly invested with sub-equal hairs; basal one-third almost hairless. Sub-marginal, marginal, post-marginal and stigmal veins related respectively in length as 14 : 7 : 7 : 4. Sub-marginal vein with 14 or 15 setae; sub-marginal cell with a proximal row of 11 or 12 setae and distally 16 irregularly arranged setae. Marginal vein with 9 to 11 macrochaetae along extreme costal edge; macrochaetae of post-marginal vein diminish in size distally and merge into ordinary marginal bristles. Stigmal vein with 4 annuli (Fig. 3 B).

Hind wing nearly four times longer than broad; average length 1·32 mm.,

[1] The apices of these setae vary in configuration: in some examples one or both may be slightly bifid.

breadth 0·35 mm. Marginal vein with 4 basal setae and 2 setae at the bend followed by 2 annuli; 14 to 16 marginal setae between latter and the frenulum.

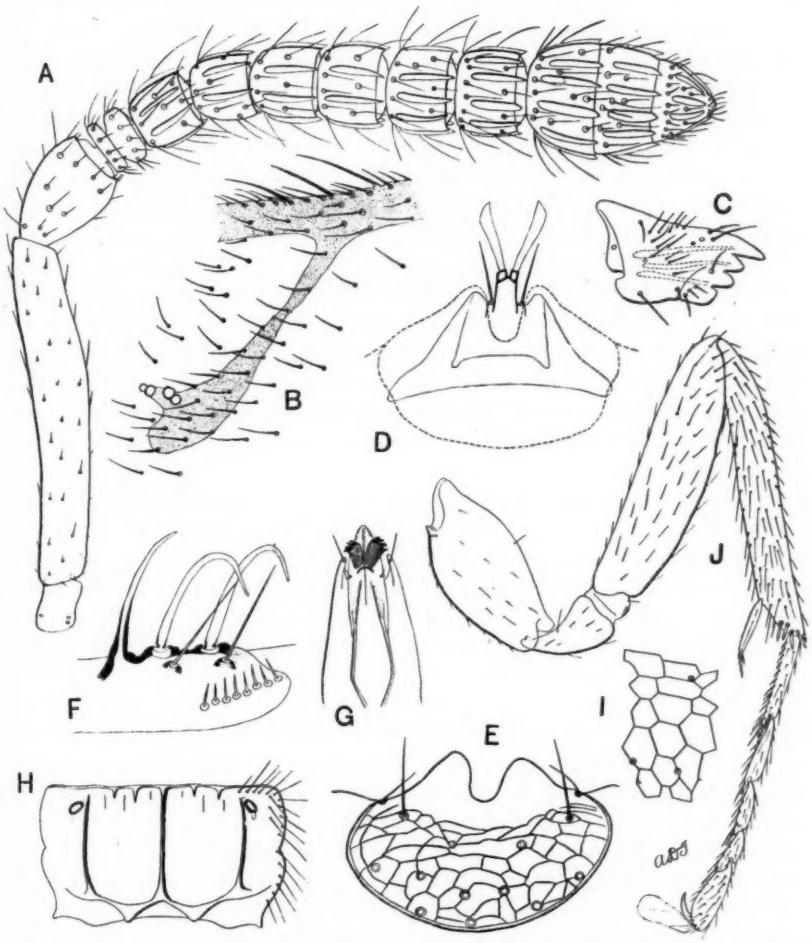

Fig. 3. *Halticoptera fuscicornis*, structural features: with the exception of G, all the parts are those of the female. A, left antenna, × 232. B, stigmal vein and adjacent portion of costal area of left fore wing, × 345. C, left mandible, × 130. D, labrum, × 300 (the broken line indicates the position of the clypeus). E, clypeus, × 300. F, frenulum of right hind wing, × 900. G, male genitalia, × 125. H, propodaeum, × 100. I, cuticular sculpturing of head from region about mid-way between orbit and median fossa, × 300. J, left fore leg, × 110.

Frenulum (Fig. 3 F) of 3 hooks; on both dorsal and ventral aspects there are 2 associated setae and, nearby, a pecten of 8 minute spines.

Legs. Fore legs (Fig. 3 J) with coxae moderately swollen, their length

related to the breadth as 3 : 2; second joint of trochanter small and weakly chitinised; femur broader and shorter than tibia, the latter bearing around its apex a girdle of 7 stout spines and a ventral bifid spur 0·11 mm. long; tarsal joints related respectively in length (measured along dorsal border) as 7 : 6 : 4 : 3 : 7; the first tarsal joint with a ventral pecten of about 14 spines increasing in length distally.

Middle legs with the coxae much smaller than preceding pair, their length related to the breadth as 8 : 7; second joint of trochanter firmly chitinised and merged with femur; femur related in length to tibia as 2 : 3; tibia with a slender acuminate ventral apical spur 0·11 mm. long; tarsal joints related respectively in length as 10 : 8 : 5 : 4 : 6.

Hind legs with coxae swollen and bearing 4 dorsal bristles, length of coxa related to breadth as 13 : 8; femur related in length to tibia as 2 : 3; tibia with a stout ventral apical spur 0·07 mm. long and adjacent thereto is a stout spine of about half that length, together with a group of 9 or 10 regularly arranged spines; tarsal joints related respectively in length as 10 : 7 : 5 : 4 : 7.

Abdomen. Petiole considerably longer than broad, its dorsal surface appearing granulate under reflected light and bearing a median longitudinal carina.

Gaster pyriform, shining, segments 1–5 smooth, 6 and 7 with the cuticle reticulated. Hairs scanty, principally on the two apical segments. First tergum nearly half total length of gaster, basally excavated to form a median fossa; second tergum slightly longer than any of remainder. Spiracles small; setigerous papillae on segment 7 projecting and surmounted by 4 hairs. Ovipositor only slightly protruding beyond apex of abdomen.

Average length 2 mm.; expanse of wings 3·25 mm.

Description of the male.

Brilliant metallic green usually with a bluish reflection, highest on the head. Eyes dark chocolate; ocelli pale translucent brown. Mandibles yellow, teeth brown; swollen apices of maxillary palpi yellow. Antennae with scape pale yellow inclining to ochreous or light fuscous distally and with the basal sub-joint smoky brown; pedicel ochreous with the base fuscous brown; funiculus ochreous, club wholly or mainly fuscous. Legs varying from amber-yellow to pale smoky ochreous especially the tibiae; coxae dark metallic green or blue-green; fifth tarsal joint and claws of first pair fuscous, of second and third pairs brown-black. Tegulae brown; wings hyaline with the veins pale yellow or inclining to a very pale fuscous tinge. Pedicel black; abdomen smooth and shining, darker than the thorax; genitalia dark fuscous.

The male is similar in size, or slightly smaller than the female but can be readily distinguished by its brilliant green colour and the pale antennae.

Apart from the genitalia (Fig. 3 G), the male differs from the female in certain small features of external structure. The most conspicuous secondary sexual characters are afforded by the maxillae which, as in other members

of the genus, differ from those of the female in the greater breadth of the stipes and in the two terminal joints of the palpi being inflated to form a conspicuous ovoid vesicle.

The reticular cuticular sculpture is more deeply impressed, especially over the head, and its cells are smaller. The first funicular joint of the antenna is shorter than any of the others and in the legs there are small differences in the relative lengths of the tarsal joints as compared with the female, viz. first legs 8 : 5 : 4 : 3 : 7, second legs 10 : 7 : 5 : 4 : 7 and third legs 9 : 7 : 5 : 4 : 8. In the fore wings there are slightly fewer setae in the sub-marginal cell, the proximal row consisting of about 10 setae and the irregularly disposed distal group consists of 8 or 9 setae.

II. **Rhoptromeris eucera** Htg.

Rhoptromeris Först. is a sub-genus of *Eucoila* (*Eucoela*) Westw., an extensive genus of the family Figitidae. Species of *Rhoptromeris* are distinguishable from other representatives of *Eucoila* by the club of the antenna being 7-jointed in the female and by the fourth or fifth antennal joint in the male being usually conspicuously enlarged. Like other members of the Eucoilinae the species are very characteristic parasites of cyclorrhaphous dipterous larvae. With regard to the hosts of *Eucoila* and its sub-genera, it may be mentioned that *E.* (s.g. *Eucoila*) *keilini* Kieff. is recorded by Keilin and Pluvinel (1913) as a parasite of *Pegomyia winthemi* Meig. found living in fungi in the forest of Fontainbleau. The species *fungicola* Kieff., *agaricola* Thoms. and *schmidti* Gir. are mentioned by Kieffer as being obtained from fungi where they presumably parasitise mycetophilid larvae. Webster and Parks record *E. hunteri* Cwfd. from *Agromyza pusilla* Meig. in Texas; Silvestri records *E. drosophilae* Kieff. from puparia of *Drosophila* in French Guinea; *E. albocincta* (Kieff.) is mentioned by Kieffer as being reared from a dipterous puparium, the species of the latter not being stated; *E. circularis* Kieff. was reared by Magretti from a gall of *Cynips kollari* in Italy and *E. siphonophorae* Ashm. is recorded by Ashmead (1887 *a*) from the aphid *Siphonophora cucurbitae* Middl. in the United States. The host of the last-mentioned species is doubtful and appears to require confirmation as it is possible that it may have been bred from a syrphid predator of the aphid in question. Of the sub-genus *Psichacra* Kieffer mentions the species *anomola* Kieff. as being reared by Carpentier from a puparium of *Pegomyia rumicis* R.D., while *impatiens* Say is a parasite of the horn-fly *Haematobia serrata* in North America and *pellaranoi* Brèthes attacks the Trypetid *Anastrepha fraterculus* Wied. in Argentina. Host-records of the sub-genus *Rhoptromeris* appear to be few in number. *R. eucera* Htg. was first recorded from *Oscinella frit* by Kurdjumov (1912) who described it as a new species *widhalmi*, which was subsequently shown by Hedicke (1923) to be synonymous with *R. eucera*. Kurdjumov's specimens, it may be added, were bred by Baranov in Russia. *R. eucera* (var. *tristis*) has also been recorded by Meyer from *O. frit* in Germany. In England this species

is abundant and in the Harpenden district it is the most important parasite of its host. It is also common in the Oxford and Leeds districts as is evidenced in collections of parasites reared by Messrs Cunliffe and Taylor. Dr Cunliffe informs me that he first reared it from *Oscinella frit* in 1919.

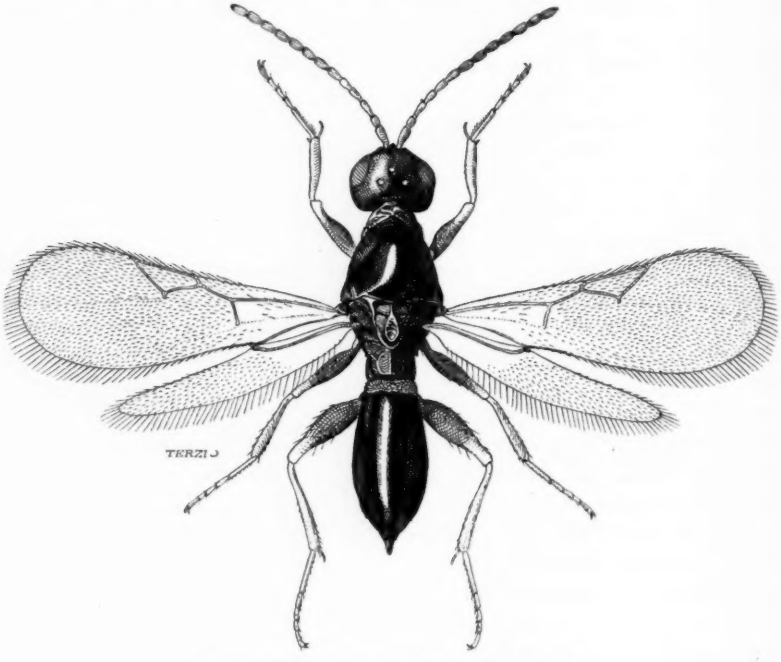

Fig. 4. *Rhoptromeris eucera* Htg., female, ×40.

Description of the female (Fig. 4).

Coloration. Shining black, inclining to brown-black ventrally; hairs cream colour on scutellum and around the junction of thorax and abdomen, elsewhere they are mostly ashy white. Antennae with the first five joints ochreous brown or, in some examples, with the scape and pedicel dark brown; sixth joint varying from brown to dark fuscous; remaining joints very dark fuscous or black. Mandibles brown. Legs ochreous brown with the coxae dark brown; femora broadly suffused with fuscous brown or entirely of that colour; fifth joint of tarsi fuscous, in some examples the third and fourth joints are a paler shade of the same colour. Wings hyaline; veins pale yellowish brown; tegulae dark brown. Terebra ochreous brown.

Head. The whole surface is smooth, with very few hairs. Frontal aspect (Fig. 5) almost circular in outline, the breadth related to the length as 32 : 35. Lateral aspect ovoid, rather longer than deep (as 26 : 21). Eyes rather small, scarcely protruding and separated by a distance rather more than half the

maximum frontal width of the head. Ocelli disposed as an equilateral triangle. Toruli circular, 0·05 mm. in diameter; separated by about their own diameter and by the same distance from the orbits. Tentorial foramina conspicuous, 0·1 mm. apart. Clypeus merged with frons; its projecting frontal margin of thin chitin and provided with setae as shown in Fig. 5. Labrum (Fig. 6 L) minute, 0·02 mm. broad, membranous and clothed with very fine hairs.

Antennae (Fig. 6 A). Length 1·1 mm., the joints related in length as 8 : 5 : 7 : 6 : 4 : 4 : 7 : 7 : 7 : 7 : 7 : 7 : 8. Sensoria present from the seventh joint upwards.

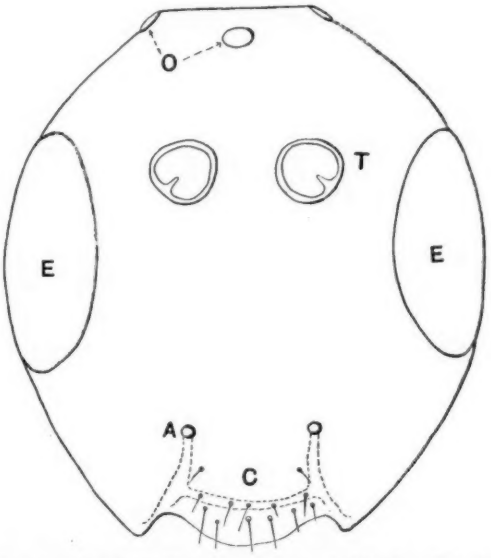

Fig. 5. *Rhoptromeris eucera*, female: outline of frontal aspect of head, × 200. *A*, tentorial foramen (right): *C*, clypeus; *E*, compound eye; *O*, ocelli; *T*, torulus (left).

Mouth-parts. Mandibles (Fig. 6 C and D) equal sized, quadrate, average dimensions 0·1 mm. × 0·08 mm.; right mandible with three teeth, left usually with two teeth, more rarely a third tooth present. Maxillary palpi (Fig. 6 E) with joints related in length as 28 : 20 : 15 (measured along external border); terminal joint with an elongate external apical seta, a minute internal seta and a campaniform sense organ. Labial palpi (Fig. 6 E) 2-jointed, related in length as 15 : 17.

Thorax. Pronotum (Fig. 6 I) reduced to a small saddle-shaped shield less than one-third the breadth of the head; its anterior and posterior margins strongly raised leaving a transverse furrow between them; prosternum smooth and hairless. Mesonotum smooth with a scanty row of marginal hairs on either side; scutellum (Figs. 4 and 6 H) with two basal foveae and a very prominent median pyriform raised area which usually bears five short stout

setae (in rare cases two only); the detailed sculpture and chaetotaxy of the scutellum is variable; mesosternum hairy. Metanotum small and concealed by the projecting scutellum; metasternum densely hairy (Fig. 6 J). Propodaeum (Fig. 6 J) with two dorsal longitudinal carinae, its ventral and side

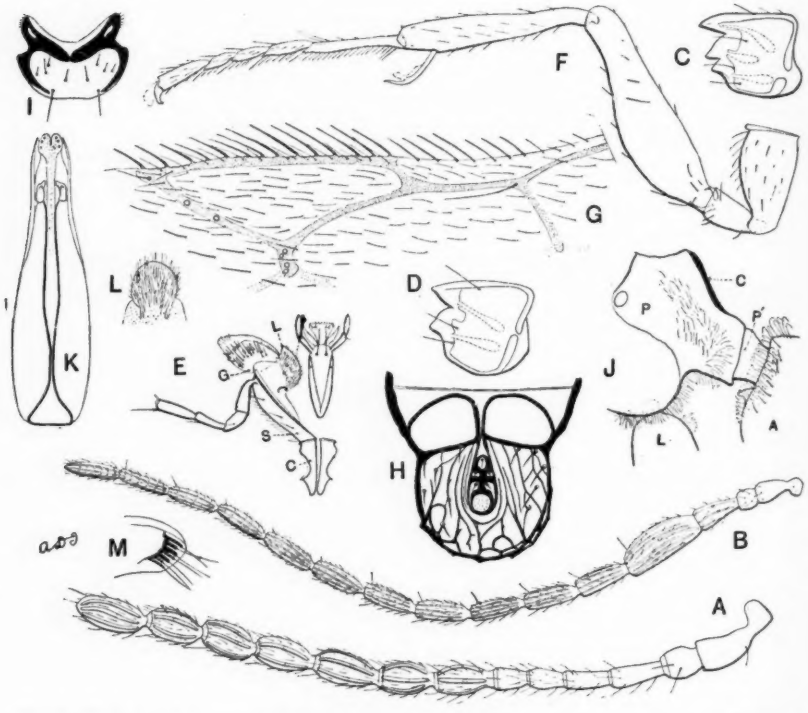

Fig. 6. *Rhopromeris eucera*, structural features: B and K refer to the male, the remaining parts are those of the female. A, left antenna (female), ×130. B, left antenna (male), ×72. C, left mandible, ×150. D, right mandible, ×150. E, labium and right maxilla, ventral aspect, ×65: *C*, cardo; *G*, galea; *L*, lacinia; *S*, stipes. F, fore leg, ×115. G, costal portion of left fore wing, ×215. H, scutellum, ×165. I, pronotum, dorsal aspect, ×120. J, junction of thorax and abdomen, lateral aspect, ×122: *A*, base of gaster; *C*, left dorsal carina of propodaeum *P*; *L*, coxa of third leg; *P*, pedicel. K, male genitalia, ×165. L, labrum, ventral aspect, ×400. M, sensory plate of last abdominal tergum, ×400.

walls hairy; spiracles circular. Fore wings (Figs. 4 and 6 G) 1·43 mm. to 1·76 mm. long and 0·60 mm. to 0·71 mm. maximum breadth. A conspicuous elongate macrochaeta at junction of sub-costal and radial veins[1]; rudiment of inter-cubital vein with four or more, rarely five, annuli; distal portion of radial vein with two annuli, very variable in position, being either adjacent to each

[1] The older vein nomenclature is followed in view of the difficulty in fixing the homologies under the Comstock-Needham system in wings with so greatly reduced and specialised venation.

other or separated by an interval; metacarpus with 12 conspicuous marginal macrochaetae[1]; traces of medius and cubitus faintly indicated. Hind wings 1·1 mm. long and 0·2 mm. broad; frenulum of three hooks, two annuli on the vein near by.

Legs. Fore legs (Fig. 6 F) with the coxa truncated proximally, length related to breadth as 11 : 7; femur bulging somewhat ventrally, related in length to tibia as 23 : 22; apex of tibia with a stout curved furcate ventral spur, 0·07 mm. long, and a minute dorsal peg-like spine; tarsal joints related in length as 11 : 5 : 4 : 3 : 6; the first tarsal joint with a ventral pecten of about 15 or 16 spines.

Middle legs are somewhat longer than fore pair; the coxa with proportions 15 : 8; femur related in length to tibia as 6 : 5; apex of tibia with a pair of ventral, slightly curved spurs of unequal length; tarsal joints as 11 : 6 : 5 : 3 : 6.

Hind legs with proportions of coxa as 20 : 9; femur shorter than the tibia, as 32 : 35; apex of tibia with a pair of ventral, slightly curved spurs of unequal length; tarsal joints as 16 : 6 : 5 : 4 : 7.

Abdomen (Fig. 6 J). Petiole hairless. Gaster smooth and shining with a conspicuous zone of irregular hooked hairs disposed around insertion of petiole and a ventral tooth below the latter. Last sternum prolonged into a simple point. Paired processes of last tergum each with a group of elongate sensory hairs as shown in Fig. 6 M.

Length (six examples) 1·4 mm. to 1·75 mm., wing-expanse 3 mm. to 3·75 mm.

Description of the male.

Shining pitchy black; antennae wholly black. Legs with coxae and trochanters black, their apices ochreous brown; femora and tibia black or brown-black with proximal and distal extremities ochreous; tarsi brown or brown-black, frequently darkening distally. Wings hyaline, veins fuscous.

The male can be readily separated from the female by the more elongate antennae (Fig. 6 B), which measure on an average 1·8 mm. long, and by the greatly enlarged fourth joint. The joints are mutually related in length as 10 : 5 : 11 : 17 : 12 : 13 : 12 : 12 : 12 : 12 : 12 : 12 : 12 : 12 : 14. The legs present no salient differences from those of the female except that the first tarsal joint is proportionately longer in all cases. The genitalia are shown in Fig. 6 K.

Average length 1 mm., wing-expanse 3 mm.

III. **Loxotropa tritoma** Thoms.

The genus *Loxotropa* Thoms. belongs to the family Diapriidae whose members are essentially parasites of dipterous larvae. Its geographical distribution is very wide, species occurring over the greater part of Europe, in North America and the West Indies, and also in Java, New Guinea, the Seychelles, Philippines, Central Africa and Egypt. Only very scanty infor-

[1] In Fig. 6 G, there should be 12 instead of 14 marginal macrochaetae.

mation exists with reference to the host preferences of the genus, and there are more records of species living in association with ants than of rearings from individual hosts. In this connection it is noteworthy that species of the closely allied genus *Basalys* have also been described from ants' nests. There is no evidence that members of either genus are parasites of the ants and, in view of the general habit of the Diapriidae parasitising dipterous larvae, it appears probable that the myrmecophilous species are parasites of Diptera living in association with ants.

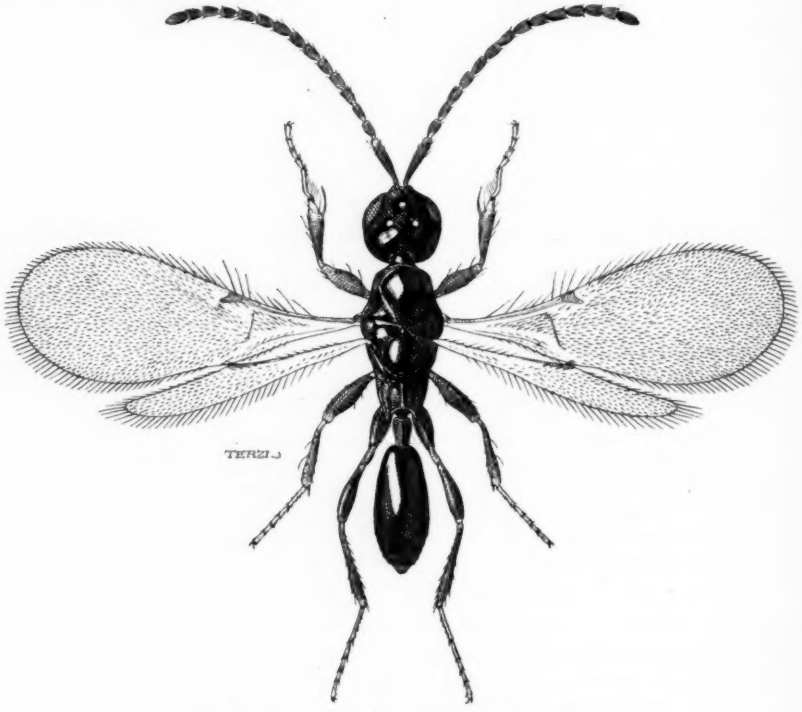

Fig. 7. *Loxotropa tritoma* Thoms., male, ×36.

Donisthorpe (1927) enumerates the following species of *Loxotropa* as being found in ants' nests: *L. donisthorpei* Kieff., *formicarum* Kieff., *subterranea* Kieff., *fuliginosi* Box, *subregonensis* Box, and *tritoma* Thoms. Kieffer (1916) also enumerates *Loxotropa steueri* Kieff. from ants' nests in Cairo and *L. ashmeadi* Kieff. from nests of the bee *Halictus pruinosus* Rob. in Virginia, U.S.A., on the authority of Brues.

Among other records, *L. pegomyiae* Brues has been reared from *Pegomyia* (*Chortophila*) *brassicae* in Minnesota (Brues, 1907). *L. flavipes* Ashm. is mentioned by Bradley (1928) as a hyperparasite bred from cocoons of a Noctuid

moth, *Papaipema maritima* Edw., in New York State; and Kieffer (1916) records *L. helicicola* Kieff. as being reared from a dipterous puparium found in a shell of *Helix aspersa* in France.

The first record of *L. tritoma* being a parasite of *Oscinella frit* is that of Meyer (1923) in Germany. Dr Cunliffe informs me that he reared four examples from that same host in the Oxford district in 1926 and the species has also been reared from *O. frit* by T. H. Taylor in the Leeds district. This insect does not appear to be an important parasite of frit-fly and in the Harpenden district only 15 examples were bred during 1926 and 1927, when they emerged between August 4th and August 23rd. Examples were reared by Dr Cunliffe on the following dates, July 20th–26th and August 11th, 1926; Meyer bred the insect in Germany in the month of July, Schander and Meyer record a single female on August 8th, 1923, and Thomson in his original description of the insect mentions August.

Description of the female.

Coloration. Shining black. Antennae brown-black or black, scape dark castaneous brown at its proximal and distal extremities; funicular joints dark castaneous brown, club dull black. Legs yellow-brown, femora fuscous, tibiae variably infuscated; fifth tarsal joint dark brown or black. Wings hyaline, apex of marginal vein of fore wing fuscous.

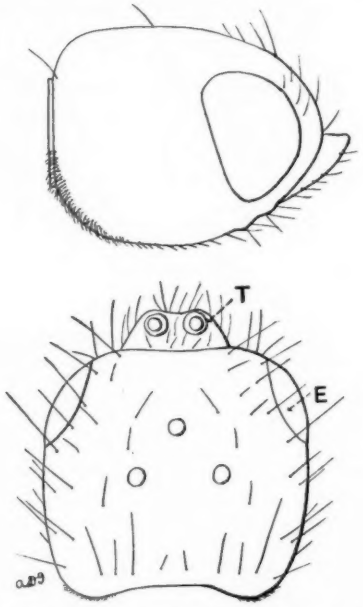

Fig. 8. *Loxotropa tritoma*, female. Above, outline of head, lateral aspect. Below, outline of head, dorsal aspect, × 114. *T*, right torulus; *E*, compound eye. (All the setae are not shown.)

Head. Cuticle smooth, markedly bristly (Fig. 8). Viewed from above cuboidal in shape with the eyes placed at the anterior angles, 0·35 mm. long from hind margin to apex of frontal process, 0·30 mm. broad; viewed laterally the head is 0·25 mm. deep. Eyes small, widely separated and located very far forward; long axis 0·15 mm. Ocelli disposed as an equilateral triangle lying wholly on the vertex; median ocellus slightly in advance of line joining hind margins of eyes. Toruli circular, separated by about their own diameter. Labrum membranous and transparent, 0·05 mm. broad, armed with prominent setae as shown in Fig. 10 C. Occiput only slightly concave, clothed ventro-laterally with closely set, short, fine hairs.

Antennae (Fig. 9 A). Length 0·9 mm. to 1·0 mm.; scape 0·25 mm. long and 0·05 mm. broad, related in length to pedicel as 3 : 1. Funicular joints related respectively in length as 22 : 15 : 15 : 15 : 15 : 16; club equal in length to scape, its joints mutually related in length as 7 : 6 : 10. Sensoria present only on club; hairs on club become shorter and more numerous ventrally.

Mouth-parts. Mandibles (Fig. 10 A) of the two sides bidentate, 0·11 mm. long and 0·05 mm. maximum breadth. Maxilla as shown in Fig. 10 D, joints of palpi related in length as 8 : 8 : 5 : 6 : 8. Labial palpi 2-jointed; basal joint greatly narrowed proximally and swollen distally; apical joint ovate.

Thorax. Mesoscutum (Fig. 10 E) smooth and almost hairless, parapsidal furrows only defined anteriorly. Scutellum broadly T-shaped, strongly elevated, side walls hairy; axillae almost hairless; tegulae rather large. Metanotum bristly, band-like and of uniform breadth (Fig. 9 C); ornamented with three well-marked longitudinal carinae; inside the lateral carina there is a conspicuous macrochaeta on either side. Propodaeum (Fig. 9 D) with the tergal region strongly raised and with a median carina; all the margins with conspicuously raised borders; pleura hairy, spiracles circular. Posteriorly the propodaeum is produced into two stout backward processes separated by a deep semicircular sinus. Fore wing (Figs 7 and 9 B) hyaline, 1·4 mm. long and 0·5 mm. broad (including marginal hairs); marginal vein related in length to wing as 11 : 28, its apex with four minute and obscure annuli; basal vein not joined with marginal. Beyond the basal vein the wing-membrane is uniformly invested with sub-equal hairs except for a small hairless tract adjacent to the apex of the marginal vein. Hind wing 1·05 mm. long and 0·22 mm. maximum breadth; marginal vein vestigial, only clearly evident at proximal and distal extremities. Frenulum of two hooks, preceded by a slender recurved seta and flanked by a pecten of 8 or 9 setae.

Legs. Fore leg (Fig. 10 F) with coxa small and globular, its length only slightly exceeding the diameter. First joint of trochanter slender, exceeding coxa in length. Femur (excluding second joint of trochanter) approximately equal in length to tibia, distinctly inflated. Tibia clavate, its length related to maximum breadth as 17 : 5. Tarsal joints related in length as 9 : 3 : 2·5 : 2 : 4, first joint markedly curved and with a pecten of closely set spines; apical spur 0·1 mm. long, bifid and ciliated; claws bristly on their bases.

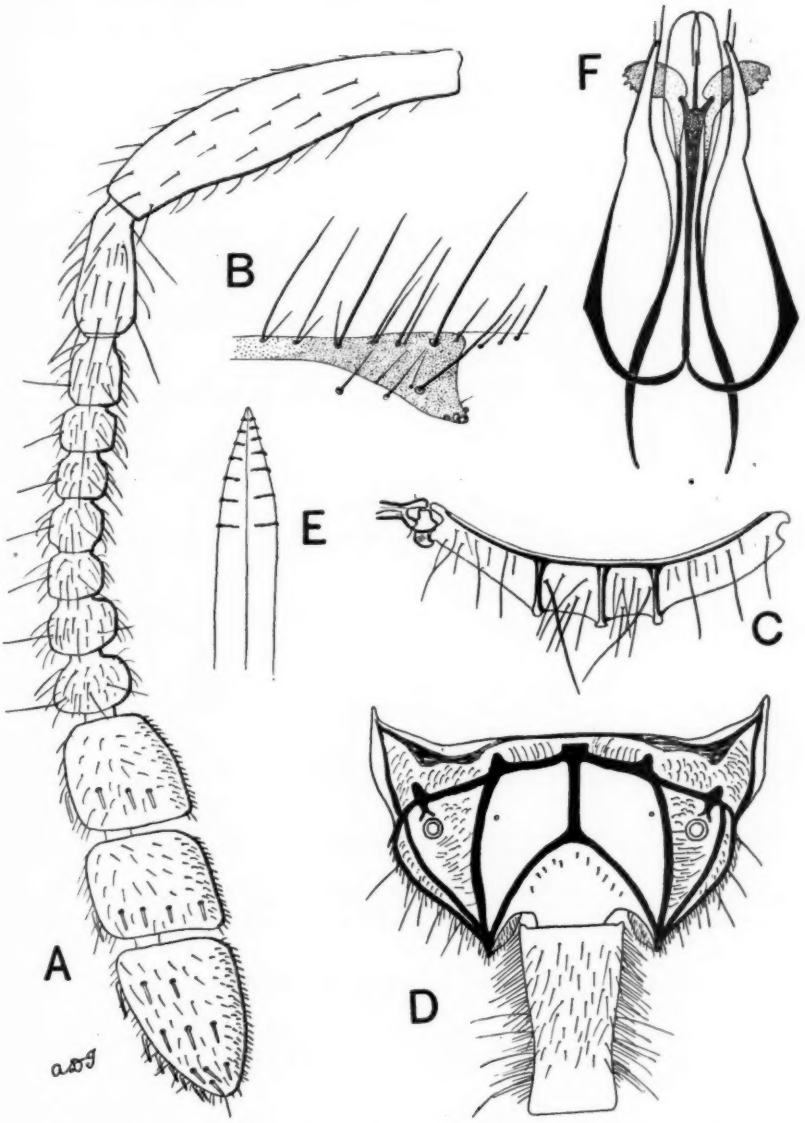

Fig. 9. *Loxotropa tritoma*, structural features: excepting F, the parts are those of the female. A, antenna (right) × 220. B, apex of marginal vein of right fore wing, × 380. C, metanotum, dorsal aspect, × 155. D, propodaeum and pedicel, dorsal aspect, × 155. E, apex of ovipositor, × 900. F, male genitalia, × 400.

Middle leg with coxa rather more elongate than fore coxa, its length related to the breadth as 3 : 2. First joint of trochanter slender, equal in length to coxa. Femur slightly shorter than tibia, its length related to maximum

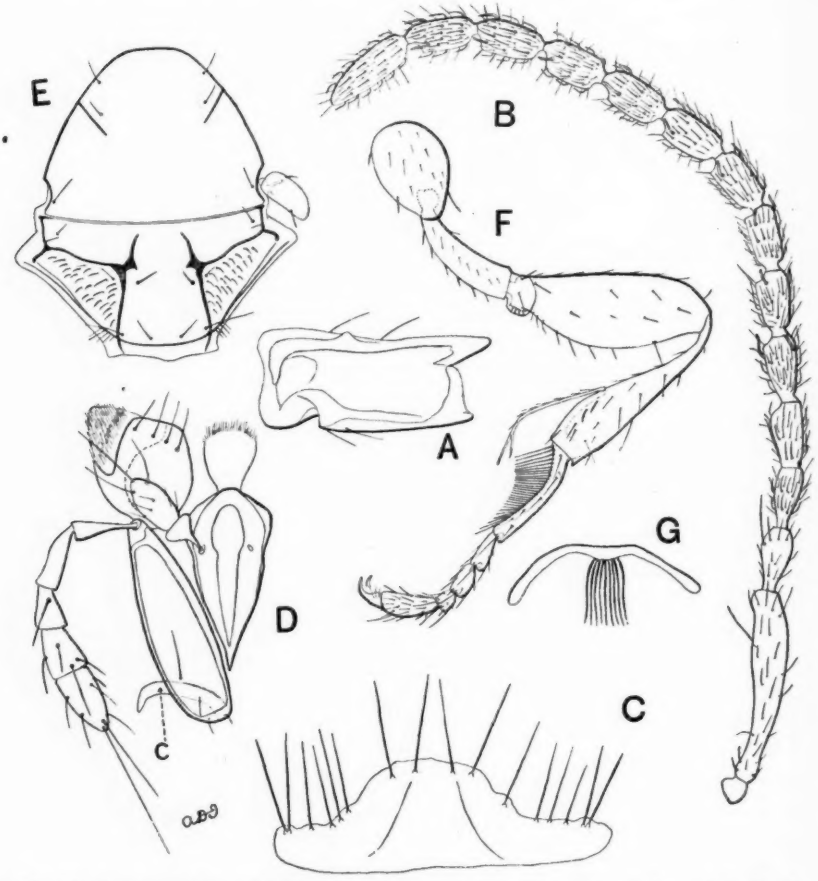

Fig. 10. *Loxotropa tritoma*, structural features: excepting B the parts are those of the female. A, mandible (right), ×345. B, right antennae (male), ×115. C, labrum, ×900. D, labium and right maxilla, ×250: *C*, cardo. E, pronotum and mesonotum, ×130. F, right fore leg, ×145. G, margin of dorsal wall of sinus at base of first tergum of gaster, showing median group of setae, ×312.

breadth as 17 : 6. Tibia clavate, its length related to maximum breadth as 5 : 1; two small apical spurs of unequal length. Tarsal joints related in length as 12 : 7 : 7 : 6 : 12.

Hind leg with coxa more elongate than that of second leg, its length related to the breadth as 17 : 7. First joint of trochanter markedly shorter than coxa.

Femur somewhat inflated, distinctly shorter than the tibia. Tibia with length related to breadth as 33 : 5, apical spur straight, 0·06 mm. long. Tarsal joints related in length as 20 : 11 : 10 : 8 : 14.

Abdomen. Pedicel 0·18 mm. long and 0·1 mm. maximum breadth; densely setose laterally, much less so dorsally and ventrally. First segment of gaster with a small sinus for reception of petiole; dorsal wall of this sinus has a strongly chitinised margin and bears a median downwardly directed tuft of regularly arranged setae of about equal length (Fig. 10 G). Apices of stylets of ovipositor each with seven minute serrations. The general surface of the gaster is smooth and for the most part hairless.

Length 2 mm., wing-expanse 3 mm.

Description of the male (Fig. 7).

In general coloration it is similar to the female. The only notable secondary sexual differences are seen in the shape of the head and the form of the antennae. The head is slightly broader than long, the breadth being related to the length as 35 : 32. The mean length of the antennae (Fig. 10 B) is 1·5 mm.; scape 0·22 mm. long, related in length to pedicel as 22 : 9; remaining joints, except the last, sub-equal in length, each joint being either equal to, or slightly longer than, the pedicel; terminal joint more elongated and rather more than half the length of the scape. In addition to ordinary hairs the flagellum bears curiously bent and forwardly directed setae resembling the sensoria, but of a much more slender form; sensoria are present on each joint of the flagellum excepting the first two joints, two sensoria being visible on each joint when viewed laterally. The relative proportions of the tarsal joints of the three pairs of legs are 12 : 4 : 3 : 3 : 6, 10 : 5 : 5 : 4 : 7, and 14 : 7 : 6 : 5 : 8. The genitalia are shown in Fig. 9 F.

Length 1·5 mm., wing-expanse 3·25 mm.

4. Remarks on the parasitism.

Observations made during 1926 and 1927 indicate that frit-fly in England is attacked by at least two species of parasites of considerable significance, viz. *Rhoptromeris eucera* and *Halticoptera fuscicornis*. These two species were also reared by Messrs Cunliffe and Taylor in the Oxford and Leeds districts respectively. Other species of apparently minor significance have been reared and among them the Proctotrypid *Loxotropa tritoma* is noteworthy. In Figs. 11 and 12 the total rearings of host and parasites are expressed graphically. The method of investigation adopted is too laborious to admit of the rearing of very large numbers of individuals or to allow of far-reaching conclusions to be drawn; nevertheless, the data obtained indicate that the host is evidently controlled by parasites to a very considerable degree.

During 1926 (Fig. 11) 27 per cent. of the insects reared were parasites which numbered 84 individuals as follows. *Rhoptromeris eucera* 24 males,

18 females; *Halticoptera fuscicornis* 9 males, 28 females; *Loxotropa tritoma* 2 males, 3 females.

During 1927 (Figs. 12, 13), when three plots were laid down, 37 per cent. of the individuals reared were parasites, viz. *Rhoptromeris eucera* 78 males,

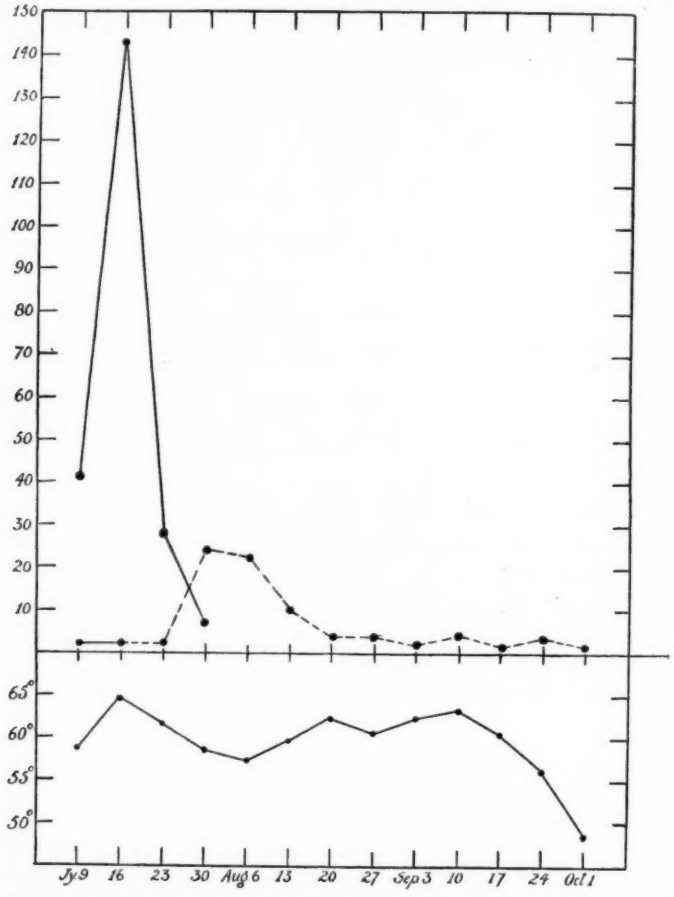

Fig. 11. Number of individuals of *Oscinella frit* and parasites (broken line), which emerged during each seven-day period at Harpenden, 1926: the first period ended on July 9th and the last period ended on October 1st. Below is shown the weekly mean temperature (°F) during that time.

70 females; *Halticoptera fuscicornis* 11 males, 29 females; *Loxotropa tritoma* 4 males, 6 females. Records of parasites reared from the plots taken individually are as follows (Fig. 13). Plot I. *H. fuscicornis* 3 males, 1 female; *R. eucera* 11 males, 11 females; *L. tritoma* 0. Plot II. *H. fuscicornis* 2 males, 16 females; *R. eucera* 36 males, 26 females; *L. tritoma* 2 males, 4 females.

Plot III. *H. fuscicornis* 6 males, 12 females; *R. eucera* 31 males, 33 females; *L. tritoma* 2 males, 2 females.

As already mentioned on p. 2 the parasitism of the host affecting the respective plots increased from 15 per cent. on Plot I to 40 per cent. on Plot II and to 54 per cent. on Plot III. This would appear to indicate that the parasites become progressively more numerous as the season advances, with the result that frit-fly, affecting late sown oats, is markedly heavier parasitised than

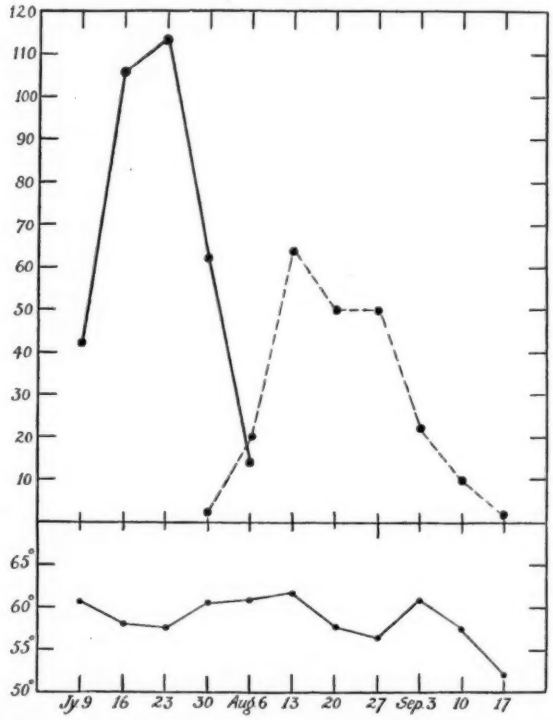

Fig. 12. Number of individuals of *Oscinella frit* and parasites (broken line), which emerged during each seven-day period at Harpenden, 1927: the first period ended on July 9th and the last period ended on September 17th. Below is shown the weekly mean temperature (°F) during that time.

when it attacks oats drilled earlier in the season. In so far as my observations extended, only a single individual parasite emerged from a host puparium and consequently each parasite represents the destruction of an individual host.

Schander and Meyer (1925) record the numbers of host and parasites reared in Germany between July 8th and August 9th, 1922. In Fig. 14 their figures have been expressed as a graph and the percentage of parasites reared

was 31·7, which is almost exactly the mean of the parasitism recorded in the present paper for the years 1926 and 1927.

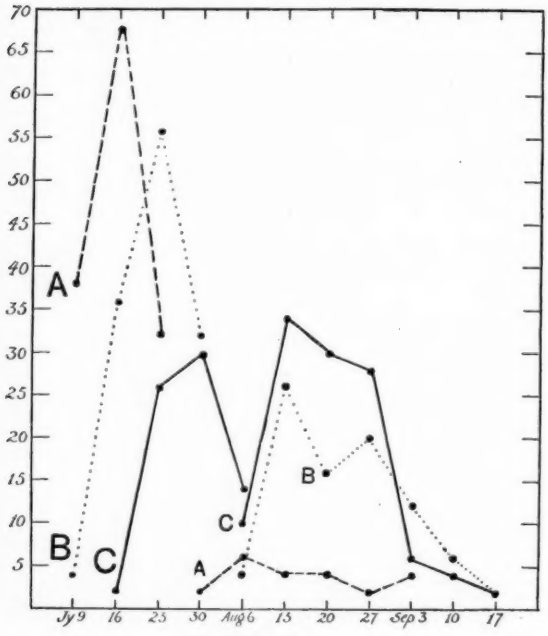

Fig. 13.

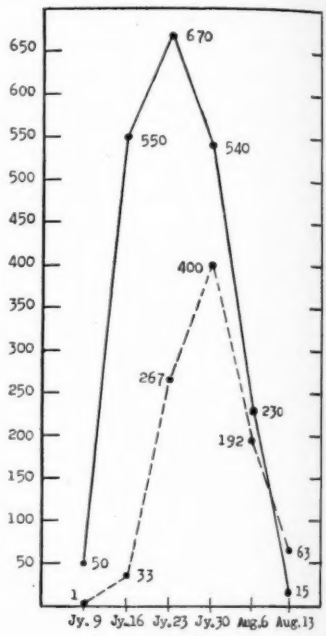

Fig. 14.

Fig. 13. Number of individuals of *Oscinella frit* and parasites, which emerged during each seven-day period at Harpenden, 1927: the first period ended on July 9th and the last period ended on September 17th. *A*, host and *a*, parasites emerging from oats sown on March 15th; *B*, *b*, the same for oats sown on March 26th; *C*, *c*, the same for oats sown on April 25th.

Fig. 14. Number of individuals of *Oscinella frit* and parasites (broken line), which emerged during each seven-day period at Landsberg a.W. (Germany), 1922: the first period ended on July 9th and the last ended on August 13th. Based upon figures given by Schander and Meyer (1925).

The following is a list of the parasites recorded as utilising *Oscinella frit* as a host. It must be pointed out that in certain cases the records are doubtful and consequently need confirmation. The most important parasites are indicated thus *.

CYNIPOIDEA.

FIGITIDAE.

1. *Cothonaspis* (Sub-gen. *Hexaplasta*) *hexatoma* Först.
(*Hexaplasta fuscipes*, Meyer, 1923.)
Germany: Meyer, 1923, 1924; Hedicke, 1923.

*2. *Eucoila* (Sub-gen. *Rhoptromeris*) *eucera* Htg.
(*Rhoptromeris widhalmi* Kurd. 1912.)
Germany: Meyer, 1923, 1924; Hedicke, 1923. Russia: Baranov, 1912. England: Cunliffe; Imms.

CHALCIDOIDEA.

MISCOGASTERIDAE.

*3. *Halticoptera fuscicornis* (Walk.).
(*Dicyclus fuscicornis* Walk. 1833.)
England: Cunliffe, 1921; Imms.

4. *Halticoptera petiolata* Thoms.
Germany: Meyer, 1923; Schander and Meyer, 1925.

*5. *Halticoptera suilius* (Walk.).
(*Miscogaster suilius* Walk. 1833: *Pachylarthrus suilius* Walk. 1846.)
Germany: Meyer, 1923, 1924; Schander and Meyer, 1925.
It appears doubtful whether this species is one distinct from *H. fuscicornis* and Dr Waterston informs me that the name *suilius* may prove to have been given to the male of *H. fuscicornis*.

6. *Semiotellus nigripes* Lind.
Russia: Wilhelm, 1891.

PTEROMALIDAE.

7. *Stenomalus micans* (Ol.).
(*Pteromalus micans* Ol.)
Russia: Rorig, 1893; Schesterikov, 1910.

8. *Pteromalus puparum* L.
Russia: Rorig, 1893.

*9. *Trichomalus cristatus* (Först.).
(*Pteromalus cristatus* Först. 1841; *Trichomalus frontalis* Thoms. 1878.)
Russia: Baranov, 1912. Germany: Meyer, 1923, 1924; Schander and Meyer, 1925.

10. *Merisus intermedius* Lind.
Russia: Wilhelm, 1891.

11. *Polycystus oscinidis* Kurd.
Russia: Mokrzecki, 1913.

12. *Callitula bicolor* Spin.
England: Imms. The first record from this host.

EULOPHIDAE.

13. *Neochrysocharis immaculatus* Kurd.
Poland: Kurdjumov, 1912.

MYMARIDAE.

14. *Gonatocerus sulphuripes* (Först.).
(*Rachistus sulphuripes* Först. 1847.)
Germany: Schander and Meyer, 1925.

ICHNEUMONOIDEA.

BRACONIDAE.

15. *Sigalphus caudatus* Nees.
England: Curtis, 1860. Italy: Silvestri and Grandi, 1911. Russia: Rorig, 1893.

16. *Gyrocampa pospelovi* Kurd.
Russia: Kurdjumov, 1912. Germany: Schander and Meyer, 1925.

17. *Chasmodon apterus* (Nees).
(*Alysia aptera* Nees *et al.*)
England: Cunliffe, 1921.

18. *Aphidius granarius* Marsh.
England: Cunliffe, 1921.

PROCTOTRYPOIDEA.

DIAPRIIDAE.

19. *Loxotropa tritoma* Thoms.
England: Cunliffe; Imms. Germany: Meyer, 1923; Schander and Meyer, 1925.

It is noteworthy that no observations have been recorded in North America respecting the parasitisation of frit-fly on that continent. According to Aldrich (1920), this insect "appears to be freely parasitised by minute Hymenoptera, but observations have not as yet excluded all doubt in any case."

During 1926 the mean temperature over the period covered by the observations was markedly higher than for the corresponding period in the following year. The maximum emergence of frit-fly in rearing cages took place during the seven-day period ending July 16th. This fact is of considerable interest in relation with field observations made by Cunliffe, who states that during the last six years the second generation of flies appeared in its maximum numbers about July 15th each season (Cunliffe, 1929). During 1927 the mean temperature at Harpenden was lower than in 1926, but this feature appeared to have little effect upon the time of emergence of the flies, as will be seen from Fig. 13. In this connection reference to Fig. 14 shows how closely the period of maximum emergence of flies reared by Schander and Meyer in Germany is in accordance with Cunliffe's field observations in England.

5. SUMMARY OF CONCLUSIONS.

The stem infestation of *Oscinella frit* in England is attacked by at least two species of hymenopterous parasites of considerable importance. The species are *Halticoptera fuscicornis* Walk. (Chalcidoidea) and *Rhoptromeris eucera* Htg. (Cynipoidea). It is also parasitised to a much lesser degree by *Loxotropa tritoma* Thoms. (Proctotrypoidea). These three species are described and figured in detail.

The three species named above have been reared by the writer from host puparia obtained in the Harpenden district; they have also been reared in the Leeds and Oxford districts by other observers. They attack the host in the larval stage of the latter and emerge from the puparia. All three species are characteristic parasites of cyclorrhaphous Diptera.

In the Harpenden district a parasitism of 27 per cent. was observed in 1926 and of 37 per cent. in 1927, *Rhoptromeris eucera* being the dominant parasite.

Evidence is brought forward which appears to indicate that the parasites, collectively, become more abundant as the season advances, with the result that *Oscinella frit* affecting late sown oats suffers markedly heavier parasitisation than when it attacks oats drilled earlier in the season.

The time of maximum emergence of *Oscinella frit* in out-of-door rearing cages during the years 1926 and 1927 coincided very closely with its period of greatest abundance in the field, as recorded by Cunliffe.

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(*MS. received for publication* 17. VII. 1929.—Ed.)

A NEW VARIETY OF *HARMOSTOMUM OPISTHOTRIAS* FROM THE NORTH AMERICAN OPOSSUM, *DIDELPHYS VIRGINIANA*, WITH A DISCUSSION OF ITS POSSIBLE BEARING ON THE ORIGIN OF ITS HOST.

By L. M. DICKERSON.

Research Fellow in the Miller School of Biology and the Blandy Experimental Farm, University of Virginia.

(With 3 Text-figures.)

PART I. THE PARASITE.

Introduction.

Several specimens of a small trematode were found in the lower end of the small intestine of an opossum, *Didelphys virginiana*. An attempt to identify this form has brought out some interesting suggestions which appear to be worth discussing at this time, although the evidence available is not sufficient to permit of valid generalisations. This paper deals with (*a*) the identification and description of this parasite for which I propose tentatively the name *Harmostomum* (*Harmostomum*) *opisthotrias* var. *virginiana* n.var., and (*b*) a discussion of the problem of host-parasite relations in the genus *Didelphys* and its bearing on problems of the origin and distribution of *D. virginiana*.

Trematodes similar to or identical with *H. opisthotrias* var. *virginiana* have been collected by workers in the United States Department of Agriculture from opossums captured in Virginia, but no description has been published reporting this species from a North American host.

The writer wishes to express his appreciation for assistance in this investigation to Dr Bruce D. Reynolds, of the University of Virginia, under whose direction the work was done; to Dr E. W. Price of the Bureau of Animal Industry and to Dr George R. La Rue of the University of Michigan for assistance in identifications and for helpful criticisms. Dr Carl G. Hartman of the Carnegie Institution of Washington, Dr W. B. Bell of the Biological Survey, Dr M. C. Hall of the Bureau of Animal Industry, and Dr G. S. Miller of the United States Museum of Natural History have also contributed useful reference material or specific information.

Historical.

The genus *Harmostomum* was established by Braun in 1899, all of these forms previously known having been placed in the more or less heterogeneous group *Distomum*. Braun (1899) described the genus on the basis of characteristics possessed by the following representatives: *Distomum leptostomum*

Ollson, *Distomum spinulosum* Hoffman, and *Distomum opisthotrias* Lutz. In describing the genus he used the name *Harmostomum* and designated *H. leptostomum* Ollson as the type species. In 1925 G. Wittenberg published a monograph on the sub-family Harmostominae, clearing up some disputed points and definitely grouping the forms into sub-genera and tribes. This work has been brought up to date and the keys revised by Werby (1928) to include several species described since Wittenberg's work was published. Werby (1928) also describes a new species from the Western Robin, *Planesticus migratorius propinquus* Ridgway, of North America, the first time a member of this genus had been reported from this continent. More recently Guberlet (1928) has described a new species from Hawaiian poultry.

One criticism offered by Werby (1928) of Wittenberg's work, and which was mentioned by Guberlet (1928), is emphasised by a study of the form discussed in this paper; namely, that arbitrary measurements are unsatisfactory means of separating groups and species. In view of the large amount of variation in the size and maturity of the individuals which have been reported in this group, it is apparent that relative measurements would be more reliable criteria. For instance, the wide variation in the size of the suckers makes a key based on such characters very unreliable for identifying such a form as *H. opisthotrias.*

H. opisthotrias was first reported as *Distomum opisthotrias* by Lutz in 1895. His specimens were obtained from two male opossums, *Didelphys aurita* Wied. (now recognised as *Didelphys marsupialis aurita* Wied.), captured near São Paulo, Southern Brazil. Braun (1899), in his paper, gives a new description based not upon Lutz's material but upon new material from the same region. Wittenberg gives a tabular comparison of the two descriptions in his monograph. Part of this table has been reproduced here (Table I) and extended to include *H. opisthotrias* var. *virginiana.* I would note here what appears to be an error in reading Lutz's paper.

In his German translation Lutz specifically states that the uterus may extend out over the ventral sucker, whereas Wittenberg quotes him as reporting it bending behind the ventral sucker and emphasises this as one of the differences between Lutz's and Brauns' descriptions. The text of Lutz's statement of this characteristic is as follows:

"Die Umschlagstelle ist in der Nähe des Bauchsaugnapfes, über den sich einzelne Schlingen hinaus erstrecken können."

In his drawings Lutz is not consistent with respect to this character; for in his diagrammatic drawing of the ventral view of the animal he represents the uterus extending only to the ventral sucker, while in his more detailed drawing of a sagittal section he represents a loop of the uterus anterior to the ventral sucker.

Material.

The new variety is described from specimens taken from two opossums (♀ and ♂) captured near Charlottesville, Va., in February and December, 1928. The female host yielded 25 specimens in addition to a few that were lost; the male host contained 34 adult trematodes. By rapid work in dissecting the

Table I. *A comparison of two descriptions of* H. opisthotrias *and that of* H. opisthotrias *var.* virginiana *n.var.*

Organism ...	*Harmostomum* (*Harmostomum*) *opisthotrias* Lutz		*H.* (*H.*) *opisthotrias* var. *virginiana*
Author ...	Braun	Lutz	Dickerson
Body size	6–7 mm. × 0·6–1·0 mm.	4·0 mm. × 0·9–1·1 mm.	1·945 mm. × 0·368 mm.
Arming	None	Present	Rudimentary
Body form	Fore end truncate ("abgestutzt")	Mostly oval but nearly cylindrical	Oval in cross-section. Ends rounded
Oral sucker	0·46 mm. × 0·66 mm.	ca. 0·3–0·4 mm.	0·259 mm. × 0·285 mm.
Ventral sucker	0·6 mm.	—	0·167 mm. × 0·198 mm.
Position of the ventral sucker	Divides the body in the prop. 1 : 3	—	Divides the body in the prop. 1 : 3
Pharynx	0·26–0·3 mm.	—	0·127 mm. × 0·109 mm.
Uterus	The bending extends even to the pharynx	*A single loop may extend over the ventral sucker	Extends in front of the ventral sucker
Beginning of the vitellaria	Back of the ventral sucker	Back of the ventral sucker	Back of the ventral sucker
Testes: Ant.	0·416 mm.	—	0·186 mm.
Post.	0·312 mm.	—	0·177 mm.
Ovary	0·187–0·312 mm.	—	0·091 mm. (short diam.)
Eggs	0·014 mm. × 0·027 mm.	—	0·016 mm. × 0·031 mm.

* Corrected to agree with the German translation of Lutz's paper.

host and the use of warm saline medium I was able to study some of these specimens several minutes in an active condition.

DESCRIPTION.

The specimens first obtained were dead when removed from the host. They were grey in colour and crescent-shaped, the dorsal surface being convex, the ventral concave. The live specimens were cream-coloured and, being imbedded in the mucous lining of the intestine, were practically invisible but for the dark brown egg masses and the whitish genital glands which stood out against the coloured background. The infected area of the intestinal wall is usually somewhat inflamed.

In both instances the parasites were confined to the posterior 15 cm. of the small intestine and most of them were within 5 cm. of its juncture with the large intestine.

The body is somewhat tongue-shaped, with rounded ends. The posterior third tapers slightly. The body is widest at a point just anterior to the testes where it is usually slightly flattened. The cuticula of the animal is diagonally striated in such a manner as to give it a "watered silk" appearance when the animal is in motion. The forepart, particularly the lateral edges and ventral surface of the animal, is provided with blunt spines which barely project through the cuticula. They are more noticeable in sections than on the living animal or in *in toto* mounts.

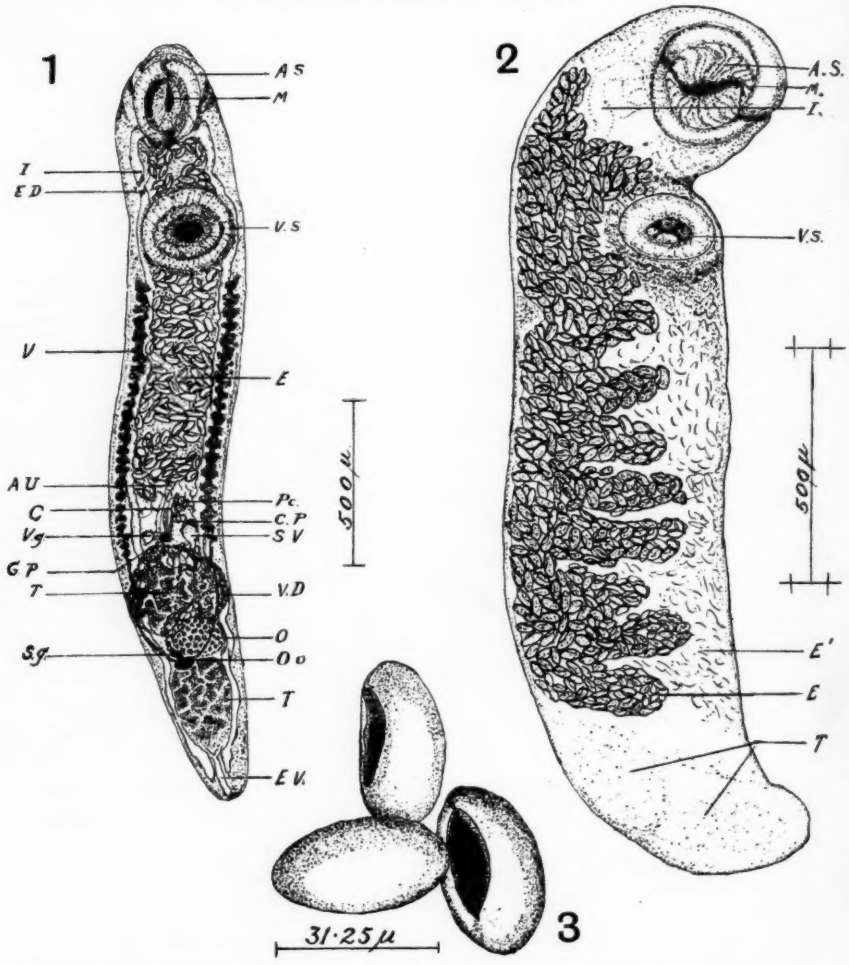

Fig. 1. Ventral view of adult *Harmostomum* (*H.*) *opisthotrias* var. *virginiana*. (Outlines with camera lucida; details somewhat diagrammatic.)[1]

Fig. 2. Right lateral view of gravid specimen showing atrophy of the sex organs.

Fig. 3. Mature eggs.

A.S. Anterior sucker.—*A.U.* Ascending loop of the uterus.—*C.* Cirrus.—*C.P.* Cirrus pouch.—*E.* Eggs (in the descending uterus).—*E'.* Eggs (in the ascending uterus).—*E.D.* Excretory duct crossing under the intestine.—*E.V.* Excretory vesicle.—*G.P.* Genital pore.—*I.* Intestine.—*M.* Mouth.—*O.* Ovary.—*Oo.* Ootype and yolk reservoir.—*Pc.* Cells of the prostate gland.—*Sg.* Shell gland.—*S.V.* Seminal vessicle.—*T.* Testis.—*V.* Vitellaria.—*V.D.* Vitellarian duct.—*Vg.* Vagina.—*V.S.* Ventral sucker.

[1] The structure of this organism is such that in a drawing on the scale used here, details cannot be presented clearly enough to show more than the general location and relative position of organs. Many of the details will have to be worked out on larval material and such material is not readily available.

The animals average 1·945 mm. (1·535–2·541 mm.) in length by 0·368 mm. (0·314–0·450 mm.) in breadth at the level of the ventral sucker. The curvature of the fixed animal is usually most pronounced at the widened and somewhat flattened area just anterior to the genital pore. The anterior sucker is strongly developed, oval in shape, and measures 0·259 mm. × 0·285 mm. (0·246–0·286 mm. × 0·205–0·300 mm.) in size. Its long axis and the longitudinal slit—characteristic of the sub-genus—are not quite parallel to the long axis of the body. The ventral sucker is also slightly oval in shape and somewhat smaller in size. It measures 0·167 mm. × 0·198 mm. (0·150–0·205 mm. × 0·177–0·218 mm.). The long axis of the oval of this sucker lies perpendicular to the longitudinal and dorso-ventral axes of the body. The sucker is deeper than wide, is very muscular and, in adult specimens, lies about one-third the length of the body from the anterior end. In immature forms it may divide the body equally. In rather immature specimens this sucker is sometimes directed slightly forward. This seems to be due to a strong dorsal contraction rather than to a difference in the length of the anterior and posterior walls of the sucker as reported by Lutz. The oral sucker opens into a muscular pharynx, globular in shape and varying somewhat in size, 0·109 mm. × 0·127 mm. (0·0955–0·123 mm. × 0·109–0·149 mm.), but usually with a diameter about half the long diameter of the oral sucker. Anteriorly it is connected by a very short—almost indistinguishable—gullet with the oral sucker and opens posteriorly directly into the intestine. The latter immediately branches, the diverticula being characteristically recurved anteriorly around the pharynx and progressing slightly dorsalwards to about the anterior edge of the pharynx. Here each bends acutely back and ranges downward, extending along the lateral edges into the caudal tip of the body. Here they end in blind sacs, separated by a distance approximately equal to the diameter of the short excretory vesicle. The intestinal caeca are straight except where they conform to the topography of the testes and ovary in the posterior part of the body and where they are distorted by pressure from the egg masses. In some specimens the blind ends run parallel to each other a short distance into the tip of the body. This character, however, is not constant.

The reproductive system entirely fills the space between the intestinal diverticula from a point just posterior to the pharynx to a point a little anterior to the ends of the intestinal caeca. The glands are crowded into the posterior end of this area. The testes are larger than the ovary which lies closely wedged between them. The ovary lies a little to the left of the mid-line. It is somewhat flattened antero-posteriorly and measures about 0·091 mm. (0·082–0·102 mm.) in its short diameter. The anterior testis measures 0·186 mm. (0·177–0·205 mm.) in diameter and lies with the mid-line a little to the right of its centre. The posterior testis measures 0·177 mm. (0·123–0·204 mm.) and lies nearly on the mid-line, being sometimes a little to the left. The departure of the vasa efferentia from their respective testes is obscured in the mature forms, but they appear to emerge from the ventral edge. Soon after they

join, the vas deferens enters the seminal vesicle. This is a large sac-like and much coiled organ lying anterior to the anterior testis and near the dorsal surface. The cirrus pouch is muscular, shaped like an Indian club with the large end entering the genital sinus and the small end bent over anteriorly as it tapers into a rather long ejaculatory duct. This makes a dorso-ventral loop about the middle of the body and a lateral loop to join the seminal vesicle. The prostate gland surrounds the portion of the ejaculatory duct connecting the cirrus pouch with the seminal vesicle and lies loose in the body parenchyma. The genital pore lies just anterior to the anterior testis. The cirrus enters the genital sinus at an angle to the vertical, being directed slightly posteriorly. The vagina enters from the posterior side and a little to the right.

Lauer's canal cannot be traced with certainty in adults, but apparently arises near the neck of the ascending branch of the uterus and extends dorsalwards between the ovary and the posterior testis.

The vitellaria lie outside the intestinal caeca, beginning immediately behind the ventral sucker and extending to the level of the anterior testis. The follicles are connected to ducts running on either side of the body, and ventral to the intestinal coeca, until they pass the anterior testis. From here they curve symmetrically inward until they meet in the yolk reservoir lying beneath and to the right of the ovary. The ootype and shell gland are contiguous with this organ and merge directly into the ascending branch of the uterus. The vitellarian ducts are easily traced, as they stain deeply in Delafield's haematoxylin. According to Lutz, the yolk material takes many stains readily and is intensely blackened by osmic acid. The uterus, beginning as a rather slender thick-walled organ, is filled with eggs the shells of which are nearly colourless at first, but change to light yellow in the ascending branch and dark brown in the descending branch. The ascending branch passes along the ventral surface of the body to a point about midway between the ventral sucker and the pharynx, in a contracted specimen. Here it curves back over and around the sucker and proceeds posteriorly in regular, characteristic coils nearly to the anterior edge of the anterior testis. From here it descends in a nearly complete coil to the genital sinus lying near the mid-line and a little anterior to this level. In size the eggs measure 0·016 mm. × 0·031 mm. (0·015–0·019 mm. × 0·026–0·034 mm.). They are operculated, concavo-convex in shape (Fig. 3) and are very numerous.

The Y-shaped excretory system is difficult to trace. In only one preparation—sections of an immature individual—have I succeeded in tracing it at all. The pore is terminal or slightly dorsal. The bladder is rather short, ranges upward and branches around the posterior testis, continuing its course dorsally and in general parallel to the intestines to the region where the latter curve upward in their approach to the pharynx. Here the branches pass between the intestines and apparently end, each as a blind sac, in the region between the ventral sucker and the pharynx.

That this is not just a group of immature forms of *H. opisthotrias* is evidenced by the specimen represented in Fig. 2. This individual was just passing into the large intestine. It will be noted that the vitellaria and glands have degenerated entirely, so that the animal is merely an egg mass enclosed in the body case. This specimen, stained in the same dish with an animal which made a beautiful preparation, absorbed no stain at all. Lutz describes such specimens in his paper.

No study has been made of the intermediate host problem.

DISCUSSION.

It should be emphasised again that linear measurements are more descriptive than diagnostic. The measurements given in this paper are averages based on from four to seven individuals representative of the group, followed by the maximum-minimum range represented in the accompanying average. For instance, the body length is given as 1·945 mm. (1·535–2·541 mm.). This is based on measurements—under the 2-inch objective—of four specimens representative of the group (of about 50 individuals). Many individuals, extremely contracted, measured scarcely 1 mm. in length.

In size this form more closely approaches *H.* (*H.*) *mesostomum* (Rud.) than any other species. However, in this latter species the body is fusiform with pointed ends and the vitellaria begin on a level with the middle of the ventral sucker. In these respects the form under consideration agrees with the two descriptions given for *H. opisthotrias*. It is evident from the comparison made in Table I that it agrees exactly with these descriptions with regard to the position and extent of the organs.

The chief differences, then, are in the consistently smaller body size and the slightly larger egg size of the smaller form. Within the descending loop of the uterus of a single individual eggs may be found representing the entire range of variation given. Much of this seems to be due to pressure, as many of the longer eggs are either narrower or of less depth than the shorter ones. Frequently, even in otherwise unshrunken mounts, the eggs show distortion which is clearly due to contact with neighbouring eggs.

The size ratio between certain organs seems to be more constant than body size. In this form the greatest diameter of the anterior testis very closely approximates the mean of the two diameters of the ventral sucker. The correlation is even closer in individual comparisons than appears in the measurements given here. It is so marked that under the microscope the organs appear to be of equal size. This relationship is noticeable at once if measurements are made on a number of specimens. Although Lutz gives no such measurements or proportions in his paper, in his diagram he represents both the ventral sucker and the anterior testis as circular in section and approximately the same size.

If a section of the intestine on which the parasites are attached be removed to warm saline medium immediately after the death of the host, the

trematodes may be seen to move about actively, though aimlessly, as though seeking a more favourable place of attachment. They become inactive after a few minutes, but for some time they may repeatedly extend and contract themselves over a distance fully twice the length of the average fixed specimen. The movement is too rapid for accurate measurement. Although numerous attempts were made to fix the animals in a distended condition (they anchored themselves by the ventral sucker and extended both the anterior and posterior portions of the body simultaneously) none was wholly successful. I am convinced, therefore, that much of the variation in body-length is due to differences in the degree of contraction at the moment of fixation. The body is also moved laterally and vertically.

It is evident that more data are needed, particularly on larval stages, to satisfactorily classify the members of this genus. Others with whom I have discussed the evidence—notably Dr E. W. Price of the Bureau of Animal Industry—favour considering this a new species, on the grounds that varieties are difficult to key and are more difficult to dispose of than species if additional data make revision advisable. To me it seems a better plan to place both generic and specific determinations on more fundamental and constant characters than are the linear dimensions in this group. Therefore, and because the structure, organisation and relative position and size of the organs of the animal are in harmony with existing descriptions, I regard it as a new variety.

PART II. THE HOST.

Lutz in 1895 reported the trematode *Distomum opisthotrias* from the opossum *Didelphys aurita* of southern Brazil. The occurrence of a form so similar to this in a North American *Didelphys* naturally raises the question of the origin and distribution of both parasite and host.

Metcalf (1923), in his discussion of the origin and distribution of the Anura, directs attention to the value of host-parasite relationship studies in connection with the solution of problems of the origin and distribution of the fauna of the world. He presents strong evidence in favour of the existence, in comparatively recent geological time, of intercontinental land connections in the southern hemisphere. He also cites some evidence to the effect that in the distribution of the land animals there has been a migration northward from South America during the latest Tertiary time. The more generally accepted view is that advanced by Dr W. D. Matthew (1915) that such dispersal in recent geologic times has been from Holarctic centres chiefly. That such southern connections existed at some time seems indicated particularly by the bio-geographical evidence. The question as to whether they existed in Mesozoic time only or extended into Tertiary time is still not clear. According to Chamberlain and Salisbury (1909) the opossums did not appear until the Eocene. In view of this, it would appear that this group not only holds a position of strategic importance but offers at the same time an opportunity for effective research.

Although a considerable amount of taxonomic work on the opossums has been accomplished, and there are in progress now numerous studies on the neurology, histology and embryology of these marsupials, their parasites are not well known. Allen (1902), in his synopsis of the genus *Didelphys*, lists 19 species and sub-species. Of these, four are known to occur in the United States. *D. virginiana* and *D. pigra* Bangs are characteristically of the United States. *D. mesamericana* Oken.—also known as *D. marsupialis*—is regarded by Allen (1902) as a composite group which, if a sufficient amount of material were studied, might be grouped into definite sub-species. His sub-species *D. m. texensis* is described from this group and its range overlaps that of *D. virginiana* in Texas. The others are from Mexico, Panama, Central America and South America. To the latter group we may add *D. marsupialis particeps*, reported by Goldman (1917) from Panama, and three fossil species, *D. abrupta*, *D. perplana* and *D. biforata*, reported from Argentina by Ameghino (1904). Allen (1902) divides the genus *Didelphys* into two groups. The *Marsupialis* group, containing 15 of the 19 species listed, are the large opossums; while the *Paraguensis* group, consisting of four species, are the small opossums characteristic of the Cordilleras, mountainous region of southern Brazil and the more southern pampas.

The range of the *Marsupialis* group is given by Allen (1902) as "...from Panama southward, extending down the Pacific coast as far, at least, as northern Peru, and also across northern South America from western Columbia to Trinidad, and southward east of the Andes, to Bolivia, northern Argentina, Uruguay, and southern Brazil. It is not, however, the same throughout this vast range, but is separable into a number of well-marked forms, the extremes of which, while widely diverse, are connected by intergradations." The *Marsupialis* group is not sharply separable from the large opossums of Mexico and the United States. Neither intergradations between the North American and South American forms nor the reverse has, as yet, been established owing to the lack of material from large portions of Central America. It is evident, however, that the relationship is close among all these forms, in comparison with their sharp differentiation from all the members of the *Paraguensis* group. Variation in coloration is especially characteristic of the opossums and over all this range, in so far as existing descriptions represent the group, seems to indicate an intergraded relationship among the larger opossums.

D. (*m.*) *aurita* (Wied.), from which Lutz described the trematode *Harmostomum opisthotrias*, differs from *D. virginiana*, in which is found *H. opisthotrias* var. *virginiana*, very markedly in colour, although there is not a great difference in size. (The body-length of the former is given as 778 mm. (745–806 mm.), head and body 417 mm. (405–426), and of the latter as 750–785 mm. (head and body about 450–520 mm.)) *D. aurita* appears to be confined to a range between northern Argentina and Bahia, Brazil on the North and the Rio do Sul on the South. It is possible that this species and *D. virginiana* may both be members of an intergraded series. Metcalf (1923)

has studied similarly graded series among the Anura of the world. He has found the members of these series to harbour parasites—particularly Opalina and certain trematodes—which when arranged in their own intergraded series indicate that their hosts have followed a certain line of dispersal. It is also possible that the opossums will be found to harbour similarly intergraded series of parasites. Likewise, other related forms may be found in other marsupial hosts either in the Americas or in other parts of the world. Other species of the genus *Harmostomum* have been reported recently from the Western Robin, *Planesticus migratorius propinquus* (Ridgway) in North America (Werby, 1928), and from poultry in Hawaii (Guberlet, 1928). The latter species, however, may have been introduced by the white or oriental peoples who have introduced different breeds of fowl into the islands in quite modern times. If such series are found it will be of interest to geographers, geologists, students of ecology, etc., as well as to the parasitologist. The problem should be kept in mind by those who have an opportunity to gather the data.

Summary.

1. A new variety of the trematode *Harmostomum opisthotrias* is reported from the North American opossum.

2. Body proportions in this genus are more constant than linear dimensions and are, therefore, considered more desirable as a basis for taxonomic distinctions.

3. The occurrence of this parasite in a host with a range as limited and a phylogenetic position as critical as that of the opossums suggests the possibility of valuable information to be obtained through host-parasite relationship studies in this group. Investigators who are working with this material should keep the problem in mind.

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(*MS. received for Publication* 17. vi. 1928.—Ed.)

FURTHER OBSERVATIONS ON THE ANATOMY AND FUNCTION OF THE PROBOSCIS OF THE BLOW-FLY, *CALLIPHORA ERYTHROCEPHALA* L.

By G. S. GRAHAM-SMITH, M.D., F.R.S.

Reader in Preventive Medicine, Cambridge.

(With Plates XIX–XXII, and 36 Text-figures.)

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I. Introduction.

In 1911 the writer (Graham-Smith, 1911) described the anatomy of the pseudotracheal membrane of the Blow-fly, *Calliphora erythrocephala*, and demonstrated the manner in which the larger particles are filtered out and rejected from a suspension on which a fly is feeding. The statements contained in that paper have been amply confirmed. Recently, further observations have been made in order to ascertain how the blow-fly folds back its labella when it desires to suck up directly into its mouth, without the intervention of the pseudotracheal filtering system, semi-solid materials, such as faeces, containing relatively large particles, and how the prestomal teeth are exposed, when required for use as cutting implements.

Although the anatomy of the proboscis of the blow-fly has been dealt with very fully in several well-known works[1], it is necessary, both in order to describe important structures, which have hitherto received little attention, and to explain the position of the parts in the different modes of feeding, to give a detailed description of the proboscis as a whole.

II. Methods.

For dissections, material from both newly emerged and older specimens was utilised, either fresh or preserved in such fluids as Carnoy's and Bouin's solutions, formalin solution and 25 per cent., 50 per cent., and absolute alcohol, or after maceration in aqueous or alcoholic sodium hydrate solutions. In order to preserve permanently the natural relationships of the chitinous parts to each other in certain specimens, illustrating different modes of feeding, proboscides in various attitudes were fixed in absolute alcohol, treated with alcoholic sodium hydrate solution, cleared and mounted in cells *without pressure*, and with as little manipulation as possible.

Serial sections, both longitudinal and transverse, were prepared from specimens fixed in various ways.

III. General anatomy of the proboscis.

The proboscis is an erectile organ consisting of three parts, named from above downwards, the *rostrum* (L.), *haustellum* (L.) and *oral disc* or *oral sucker* (L.). When at rest the parts are flexed on each other, and the whole organ is withdrawn by muscular action within the head capsule, so that it is either wholly invisible or only the distal extremities of the palps and labella are seen, when the head is viewed from the side (Fig. 13). When fully extended, the attitude in which it is usually described, the proboscis projects downwards,

[1] Unfortunately the nomenclature used by various authors differs considerably. The names used by one or other of the authors mentioned below have been retained, but it has been necessary to adopt new names for structures which have either not been named or have been insufficiently described by them. To facilitate reference the letter (L.) is placed after names used by Lowne (1892), the letter (P.) after names used by Patton and Cragg (1913), and the letter (G. 1) after names introduced by Graham-Smith (1911). The letter (G. 2) is placed after names proposed in this paper.

the three parts being nearly in a straight line[1]. The extension of the rostrum is not brought about by direct muscular action, but by the distension of the large air sacs which are contained within it. The varied movements of the distal parts of the proboscis are produced by muscular action.

The exoskeleton or integument of the proboscis consists in most parts of a thin layer of chitin secreted by a single layer of chitinogenous cells, set on a thin basement membrane. In some places the layer of chitin is thickened in accordance with structural requirements or for the attachment of muscles. Most of these thickened areas have received special names. Lying more deeply are certain strong chitinous structures which form walls for the alimentary canal, or serve to give stability to different parts of the organ or afford surfaces for muscular attachment.

IV. THE EXTERNAL FEATURES.

The external features are best studied in fresh preparations, especially from newly emerged flies, in conjunction with fixed specimens and others which have been macerated and mounted without compression, so that the relation of the parts has been disturbed as little as possible. In dried specimens great distortion is caused by shrivelling, and in fresh preparations from mature adults pigmentation and opacity cause considerable difficulty in determining many points.

The following is a description of the parts as seen with the aid of a Zeiss binocular dissecting microscope in the proboscis of the newly emerged fly before the chitin has hardened thoroughly or become deeply pigmented.

The *rostrum* (L.), or basal part of the proboscis, is cone-shaped with the base uppermost. Its wall, which consists of a tough but flexible membrane, is attached proximally to the margins of the *epistomal orifice* (P.)[2] of the head capsule, into which the proboscis can be retracted. These margins are formed in front by the *epistome* (L., P.), laterally by the *genae* (L.) or *cheeks* (P.), the regions below the eyes and external to the *facial plate* (P.), and behind by the *pars basilaris* (L.). At its distal end the wall of the rostrum is continuous with that of the haustellum.

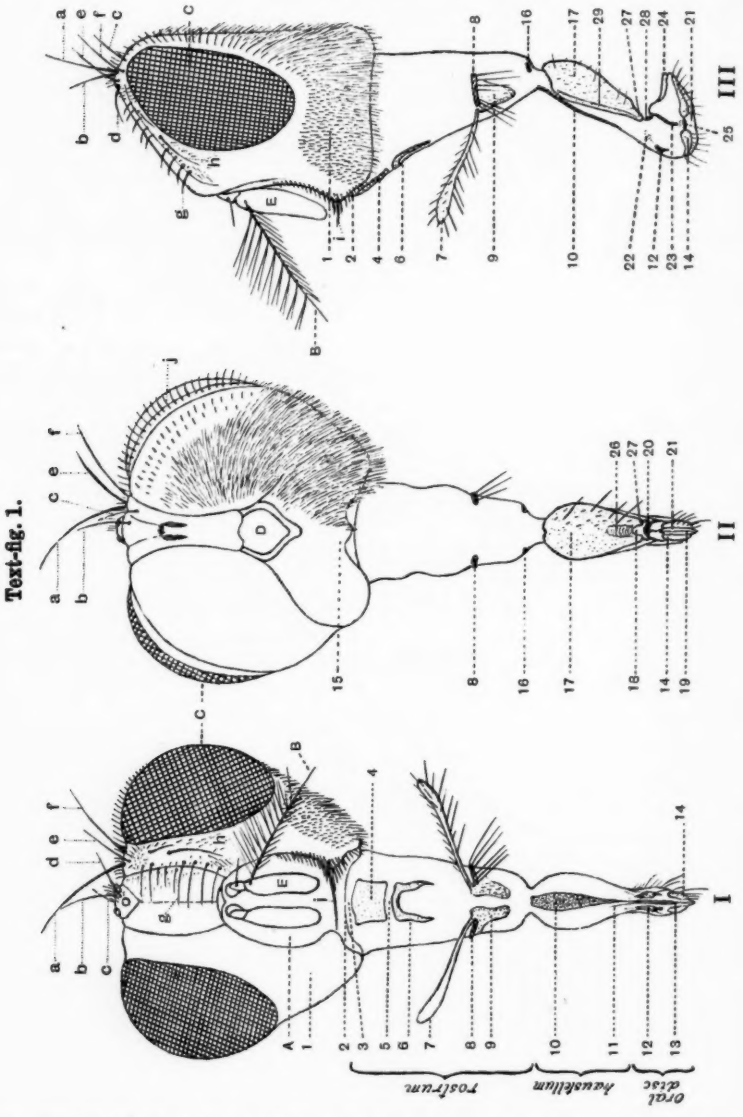
The *anterior* or *dorsal surface* of the rostrum is somewhat flattened. It articulates by a narrow syndesmosis, which forms a hinge, with the *epistome* (L., P.), a small mesial plate lying below the *facial plate*. Below the hinge is the small semi-transparent *trapezoidal plate*[3] (L., 1870) of thicker chitin.

[1] The words *anterior* or *front* and *posterior* or *back* relate to the surfaces which are directed forwards and backwards respectively, when the extended proboscis is hanging in its natural position below the head. Lowne (1870, p. 41) applies the words *dorsal* and *ventral* to these surfaces since they "assume those positions, where the organ is extended and drawn forward."

[2] The epistomal orifice "is sometimes referred to as the *buccal orifice*, a most unfortunate term, for it is some distance posterior to the structure universally known as the pharynx, and has nothing whatever to do with the mouth parts....It is also known as the *oral orifice*" (Patton and Cragg, 1913, p. 15).

[3] Lowne in a later edition (1892, p. 135) calls this the *clypeus*, a term used by some authors to indicate the face (Patton and Cragg, 1913, p. 14).

Below the trapezoidal plate a dark horse-shoe shaped mass of chitin, forming the *anterior arch* (P.) of the internally placed *fulcrum*, is visible on the surface.



The trapezoidal plate is united with the fulcrum by a strip of thin chitin, which forms a hinge. When the proboscis is withdrawn within the head capsule (Fig. 13) acute flexion occurs at both these hinges, which may be called the

superior and *inferior syndesmoses of the trapezoidal plate* (G. 2). Below the visible portions of the fulcrum there lies on either side of the middle line a triangular area of moderately thick chitin, the *palpigerous scale* (L.), supporting at its inner proximal angle a single-jointed, clavate *maxillary palp* (L.), which bears a few long, tubular chaetae and many short hairs. A large branch of the labial nerve enters each palp and distributes fibrils to the hairs, which appear to have sensory functions. The larger hairs possess "similar organs to those connected with the setae of the labial lobes" (Lowne, p. 403). The pigmentation of the palpigerous scale is deepest at its inner margin, and from its surface several short hairs arise. The upper margin of each scale extends horizontally outwards on to the lateral aspect of the rostrum as a strong pigmented *band* (L.) of chitin (= "basal ring of maxilla," Suffolk, 1869), bearing a row of eight or more long chaetae. In some diptera these bands pass round the rostrum and fuse. In the blow-fly the bands terminate in definite rounded extremities on the lateral aspects of the rostrum, but in occasional specimens indefinite central thickenings of the chitin on the posterior surface may be seen, representing the point of union. Below the scales the rostrum narrows and merges with the haustellum.

On the *lateral aspect*, apart from the chaetigerous bands, no thickened areas of chitin occur. On the *posterior aspect* no thickened areas of chitin are present, except two small, short rods, the *sesamoid sclerites* (L.), situated close to the junction with the haustellum, into which the *retractor muscles of the rostrum* are inserted.

There are no long hairs on the rostrum, except those arising from the palps, scales and bands.

The contours of the areas covered by thin integument vary greatly, depending on the degree of distension of the air sacs.

The **haustellum**, or middle part of the proboscis, is distinctly broader above and gradually narrows to its junction with the oral disc.

Along the central line of its *anterior surface* runs a dark, longitudinal

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Text-fig. 1. Views of the anterior (I), posterior (II) and lateral (III) aspects of the head of a freshly emerged specimen of *C. erythrocephala* with the proboscis extended, but the labella in apposition. In I and II the bristles are omitted on the left side. *A*. Facial plate. *B*. Arista. *C*. Compound eye. *D*. Occipital foramen. *E*. Antenna. Bristles—*a*. Inner vertical. *b*. Inner post-vertical. *c*. Outer post-vertical. *d*. Ocellar. *e*. Fronto-orbital. *f*. Outer vertical. *g*. Frontal. *h*. Lower fronto-orbital. *i*. Vibrissa and below it the Peristomal. *j*. Post-orbital.

1. Cheek or gena. *2*. Epistome. *3*. Superior syndesmosis of trapezoidal plate. *4*. Trapezoidal plate. *5*. Inferior syndesmosis of trapezoidal plate. *6*. Anterior arch of the fulcrum. *7*. Maxillary palp. *8*. Chaetigerous band of the palpigerous scale. *9*. Palpigerous scale. *10*. Labrum-epipharynx. *11*. Disco-haustellar folds. *12*. Basal sclerite of the disc. *13*. Dorsal seam. *14*. Lateral fold. *15*. Pars basilaris (L.). *16*. Sesamoid sclerite. *17*. Mentum. *18*. Semicircular sinus between the forks of the mentum. *19*. Prestomal margin. *20*. "Spring" and central process of the furca. *21*. Marginal strip. *22*. Hairs near basal sclerite of disc. *23*. Lateral process of furca. *24*. Inter-labellar membrane. *25*. Epifurca. *26*. Groove in the mentum proximal to the semicircular sinus. *27*. Fork of mentum. *28*. Mento-furcal bar. *29*. Lateral ridge of mentum.

band of chitin, broad at its proximal but narrow at its distal end. This is the anterior surface of the *labrum-epipharynx* (P.) which lies, partly at its proximal and almost wholly at its distal end, in a deep groove with strongly chitinous sides and base, the *labial gutter* (P.), and laterally is covered partially by the *disco-haustellar folds* (G. 2) of the integument. At its proximal end the anterior wall of the labrum is fused with the membranous anterior wall of the rostrum.

A heart-shaped convex plate of deeply pigmented chitin, the *mentum* (P.) or *thyroid* (L.), broadest proximally and divided by a deep *semicircular sinus* (G. 2) or indentation at its distal end, covers the whole of the ventral and the upper half of the lateral surfaces of the haustellum. The distally projecting processes of the mentum separated by the semicircular sinus are called the *forks* (P.) or *cornua* (L.). The outer strongly pigmented sides of the forks, called by Lowne the *outer supports of the cornua* (L.), incline slightly from the plane of the mentum into the substance of the haustellum, but the inner sides, or *inner supports of the cornua*, incline steeply, drawing inwards the thinner integument which unites them and thus forming a groove in the posterior surface of the haustellum, in which the posterior part of the oral disc lodges in the resting position. A shallow depression in the lower part of the mentum is a continuation of this groove. The lateral margins of the mentum are strengthened by two internal *lateral ridges* (L.), which terminate in the outer supports of the cornua. Two short, strong, slightly curved rods of chitin, the *mento-furcal bars*[1] (G. 2), articulate with the distal margins of the forks. The distal ends of the bars are lodged in fossae in the lateral processes of the *furca* (L.), an important structure situated in the oral disc. Numerous hairs arise from the external surface of the mentum.

A band of short but strong hairs is situated on the lateral aspect of the haustellum above its junction with the oral disc (Text-fig. 2, *11*).

The terminal part of the proboscis is known as the **Oral disc.** It consists of two *labella* (P.) or *oral lobes*, springing from a strong hoop of chitin, the *discal sclerite* (L.), shaped like the "frame" of a lawn-tennis racket open at its anterior end, where the splice of the handle is inserted(Text-fig. 14). The *discal sclerite* articulates with the distal end of the labial gutter of the haustellum. The discal sclerite is not visible on the surface, but a line (Text-fig. 2, I, *B*) drawn roughly parallel with the long axis of the labella from the distal extremity of the mentum to the proximal extremity of an elongated plate of pigmented chitin on the anterior surface of the disc, which may be termed the *basal sclerite of the disc* (G. 2), marks its position, and therefore the proximal limit of the oral disc. The relation of this line to the long axis of the haustellum varies from an acute angle in the resting position to a right angle when the oral disc is extended.

In the resting position the *labella* are flattened, leaf-shaped lobes projecting beyond the haustellum and lying parallel to each other. Their inner

[1] Lowne (1892, p. 142) figures these bars, but regards them as direct continuations of the forks.

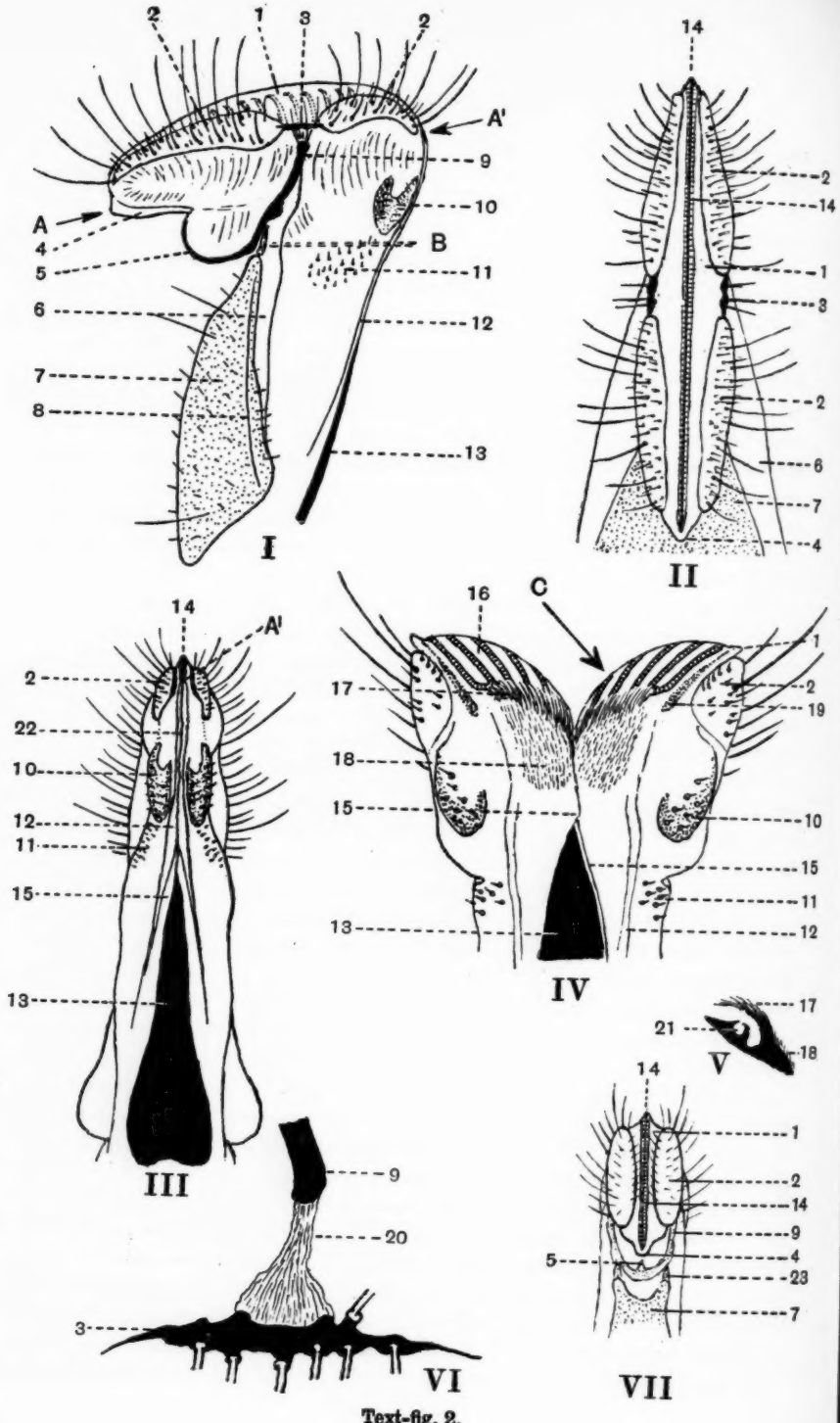
surfaces, grooved by the pseudotracheae, are in contact, and their outer surfaces are continuous proximally with the walls of the haustellum. During the act of feeding, the labella, which can be rotated outwards on the discal sclerite, are separated from each other like the leaves of a book.

In the ***resting position*** the oral disc, when *viewed from the side*, is oval with one end, the posterior, pointed. On its surface the most striking feature is the *lateral process of the furca* (L.), a strong rod of chitin lying in the outer wall of the labellum, which stretches from near the fork of the mentum obliquely forwards to within a short distance of the centre of the free, distal or prestomal margin of the disc. A well-marked fold in the integument passes from the centre of the lateral process of the furca to the anterior edge of the mentum, and indicates the position of the retractor muscle of the furca. A short distance beyond the distal end of the lateral process of the furca and at right angles to it is situated a short, indented rod of chitin, bearing macrochaetae, the *epifurca* (L.). The epifurca is connected to the furca by a strap-like band of strong moderately pigmented, but thinner chitin (Text-fig. 2, VI).

The shorter and more delicate *central process of the furca* projects downwards and backwards in a smooth curve from near the distal end of the mentum, and forms the support of the posterior wall of the disc. Beyond the central process of the furca the labella are united to each other as far as the pointed end of the oval (Text-fig. 2, I, *A*) by a thin membrane, which may be termed the *interlabellar membrane* (G. 2).

The *prestomal margin* (G. 2) of the disc, where the *free* edges of the labella are in apposition, extends in a semicircle from the "point" to the centre of the opposite end of the oral, where the *dorsal seam* (L.) commences (Text-fig. 2, I, *A*). The free or prestomal edge of the labellum is very thin and has a beaded appearance, owing to the presence in its outer wall of a series of small, quadrilateral chitinous plates. The margin is bordered by a narrow, smooth, slightly pigmented strip of the labellum, devoid of hairs, which may be called the *marginal strip* (G. 2), in which the curved distal extremities of the pseudotracheae, situated on its inner surface, may be distinguished through the substance of the labellum (Text-fig. 2, I, *1*), especially in the central area. Proximally to the marginal strip a thicker and more opaque portion of the wall of the labellum, bearing numbers of short and long sensory macrochaetae, follows the line of the margin, except in the centre where it is deflected towards the epifurca. This may be termed the *lateral fold* (G. 2). The macrochaetae on the lateral fold are the first parts of the proboscis to touch any object, and doubtless give some intimation of its nature. Beyond the anterior end of the prestomal margin (*A'*) the borders of the labella are modified to form the interlocking disco-haustellar folds, which are continuous with folds on the anterior surface of the haustellum (see p. 57). The integument covering the surface of the labellum proximal to the lateral fold bears in most places very small hairs (Text-fig. 26) and is thrown in slight folds.

Viewed from the *posterior aspect* (Text-fig. 2, VII) a vertical central line is



Text-fig. 2.

seen, along which the apposed prestomal margins of the labella meet. The line, which is bordered on each side by slight beading, caused by the small quadrilateral plates, runs along the apex of a ∧-shaped ridge, the sides of which are formed by the marginal strips. External to the ridge lie the lateral folds, from which arise the forwardly projecting macrochaetae. The interlabellar membrane is visible uniting the posterior extremities of the two labella.

Viewed from the *distal extremity* (Text-fig. 2, II) the whole length of the prestomal margin, bordered by the marginal strips, which are broadest centrally where they dip towards the epifurcas, and beyond them the marginal folds may be seen.

Viewed from the *anterior aspect* (Text-fig. 2, III) the vertical central line of the prestomal margin is seen to be continued proximally as the *dorsal seam* (L.) on to the haustellum. The seam extends to the point at which the labrum-epipharynx becomes superficial. Along the dorsal seam folds of the integument the *disco-haustellar folds* (G. 2) meet, and roof over the distal end of the labial gutter, which lies beneath them. In the resting position the basal sclerites of the disc lie parallel to the dorsal seam on each side of it, but in the feeding positions they are displaced so as to lie obliquely to the seam at the bases of the swollen labella (Text-fig. 2, IV).

The part of the inner surface of the labellum which adjoins the discal sclerite is furnished with three distinct rows of chitinous *prestomal teeth* (L.), and the rest is grooved by channels known as *pseudotracheae* (L.), which run inwards from near the prestomal margin to open between the prestomal teeth. The pseudotracheae are blind at their distal extremities, but throughout their length they communicate with the surface by means of numerous small perforations, the *interbifid grooves* (G. 1). Suction created by the pharynx draws fluid through the interbifid grooves into the pseudotracheae.

Text-fig. 2. I. Side view of part of the haustellum and the oral disc (extended, but with the labella in apposition). II. Distal surface of the oral disc, in the resting position. III. Anterior surface of the haustellum and oral disc (extended, but with labella in apposition). IV. Anterior aspect of the oral disc, with the labella distended and partly separated. V. Diagrammatic section through the surface of the labellum in the region of the common anterior collecting channel in the plane of *C*, fig. IV. VI. Attachment of furca and epifurca. VII. Posterior surface of the oral disc in the resting position.

1. Marginal strip (in I the dotted, curved lines represent the distal ends of the pseudotracheae grooving the inner surface of the labellum). *2.* Lateral fold. *3.* Epifurca. *4.* Interlabellar membrane. *5.* Central process of furca. *6.* Fold in the integument caused by the retractor muscle of the furca. *7.* Mentum. *8.* Lateral ridge of mentum. *9.* Lateral process of furca. *10.* Basal sclerite of disc. *11.* Hairs near basal sclerite of disc. *12.* Superior disco-haustellar fold. *13.* Labrum-epipharynx. *14.* Prestomal margin. *15.* Inferior disco-haustellar fold (the one on the left overlaps the one on the right). *16.* Pseudotracheal surface of the labellum. *17.* Brush. *18.* Hair tract leading to brush. *19.* Band of pigmented chitin along the inner side of the prestomal margin. *20.* Strap-like band of pigmented chitin connecting the lateral process of the furca and the epifurca. *21.* Section of common anterior collecting channel. *22.* Dorsal seam, commencing at *A′*. *23.* Mento-furcal bar.

A...*A′*. The line along which the labella are folded back in the "filtering position." *B*. Level of discal sclerite. *C*. Direction of section V.

When the fly desires to suck up a thin layer of fluid the distal portions of the labella are caused to diverge from each other, like the leaves of a book, so as to bring them into a plane lying perpendicular to the long axis of the proboscis, and in contact with the surface on which the fly is feeding, but the proximal portions remain in close contact along the line *A*...*A* (Text-figs. 3, 35). In this position only the areas of the labella furnished with pseudotracheae are applied to the fluid, which is sucked in through the interbifid grooves, and all particles too large to pass through them are filtered out and rejected. This may be termed the *filtering position* (G. 2).

While the labella are in the filtering position their marginal strips may be erected to form a "rim," transforming the oral disc into an apparatus, resembling a Büchner's funnel, with its sides and bases perforated by the interbifid grooves. Since the action of the old-fashioned cupping apparatus is simulated, this may be termed the *cupping position* (G. 2). (Text-fig. 35, III.)

By separating the labella further the prestomal teeth may be used to a slight extent, while fluid is being filtered through the pseudotracheae. This may be termed the *intermediate position* (G. 2). (Text-fig. 35, IV.)

When the fly desires to use its prestomal teeth for scraping the labella are further separated, and are folded back along the line *B*...*B*, so as to expose the prestomal teeth. In this position the filtering or pseudotracheal areas are out of action. This may be termed the *scraping position* (G. 2). (Text-fig. 35, V.)

Extreme folding back of the labella along the line of the discal sclerite, *C*...*C*, rotates both the pseudotracheal areas and the teeth outwards and upwards, so that the oral aperture or opening of the mouth at the discal sclerite can be applied directly to the food, and comparatively large objects, such as helminth ova, ingested. This may be termed the *direct-feeding position* (G. 2). (Text-fig. 35, VI.)

V. The plan of the proboscis.

In order to understand the internal anatomy of the proboscis and the functions of its various parts it is necessary to visualise the plan on which it is constructed.

The oesophagus, after traversing the brain (P.), enters the proximal part of the rostrum as a muscular tube and passes into the *pharynx* (L.), or cavity between the dorsal and ventral plates of the fulcrum. These plates, which in the resting position are in apposition, can be separated by the *dilator pharyngis muscle* in order to create suction. Beyond the distal end of the pharynx the alimentary canal is continued as a short tube strengthened by a hoop of chitin, the *hyoid sclerite* (L.), which is shaped like a signet-ring and does not afford attachment to muscles. This region has been called the *buccal cavity* (P.). Beyond the buccal cavity the canal lies (before entering the haustellum) in a short chitinous tube, called by Lowne (p. 147) the *prepharyngeal tube* (L.). Its anterior and lateral walls are formed by the inner plate (epipharynx) of the labrum-epipharynx and its posterior wall by flanges from the hypopharynx,

connected to the former by thin membrane (Text-fig. 10). In its passage through the rostrum the alimentary canal is therefore centrally situated and tubular.

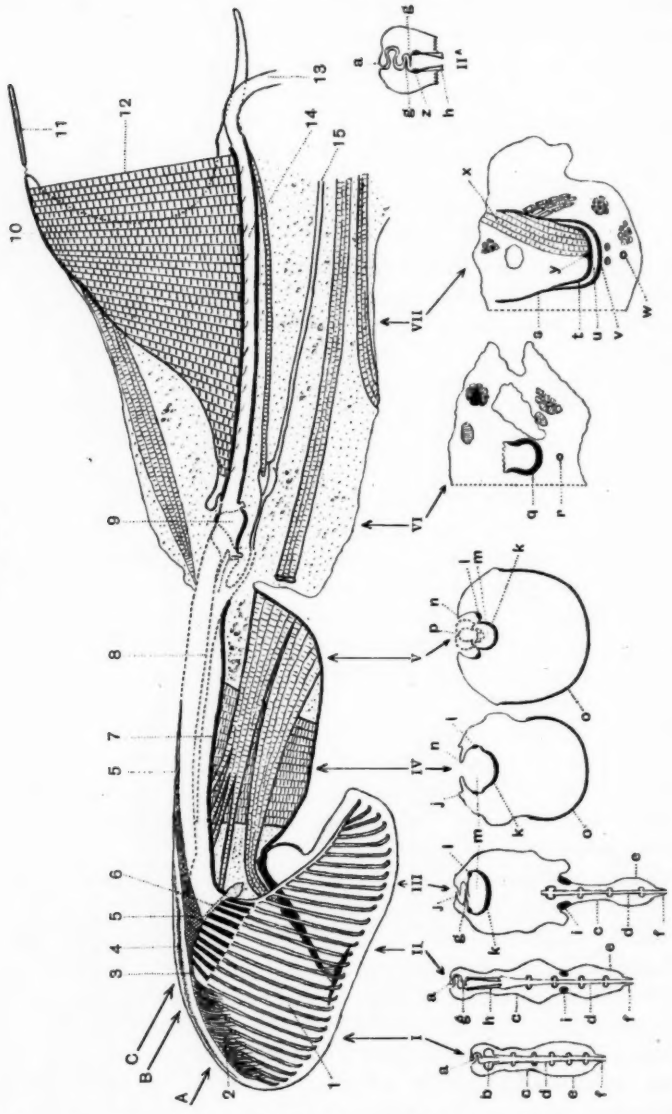
On passing out of the rostrum the character of the canal changes, and from a tube it becomes a deep groove, the *labial gutter* (P.), in the anterior surface of the haustellum. The gutter, however, is roofed over throughout, proximally by the strongly chitinous labrum-epipharynx and distally by the superior and inferior disco-haustellar folds (G. 2) of the integument, so that along its whole length the potentially open gutter functions as a tube, whatever the position of the labella[1].

The gutter is continued through the *oral aperture* (Hewitt, 1914, p. 17) in the discal sclerite into the oral disc, where it becomes a very deep fissure, the *prestomum* (L.), separating the inner surfaces of the two labella. The disco-haustellar folds, extending from the haustellum and meeting along the *dorsal seam* (L.) roof over its anterior portion. In the resting state the prestomal cavity is closed inferiorly by the apposition of the prestomal margins of the labella, but in the act of feeding the labella are widely separated.

The *superior disco-haustellar folds* (G. 2) extend from the anterior extremity of the prestomal margin (Text-fig. 2, I, III, *A'*) to the distal fourth of the haustellum, and meet along the central line to form the *dorsal seam* (L.). In the resting state the integument on the right side is folded in such a manner that in section it appears as a thin projection with a beaded margin (Text-fig. 3, *a*). This fits into a furrow in the integument on the left side. At their proximal and distal ends the folds, gradually becoming smaller, fail to interlock and fade into the integument. Under some (perhaps artificial) conditions the labella are so distended with fluid that the basal sclerites of the disc are separated and the folds, which lie internal to them, are nearly obliterated, and cease to roof over the gutter (Text-fig. 2, IV). The superior folds, which are figured and very briefly described, but not named, by Lowne, usually roof over the anterior region of the prestomum, but may fail to function if the labella are greatly distended.

The *inferior disco-haustellar folds* (G. 2) arise in the haustellum from the free edges of the paraphyses. Proximally they cover only the lateral portions of the labrum-epipharynx, but gradually they increase in size so that in the distal fourth of the haustellum they overlap each other, completely covering the extremity of the labrum-epipharynx and roofing over the gutter. Beyond the articulation of the paraphyses with the discal sclerite the folds continue, now arising from the inner margins of the cochleariform processes, and roof over the anterior region of the prestomum. Beyond the cochleariform processes the folds are continued as low projections from the inner surfaces of the labella, external to the anterior common collecting channels. From the

[1] Lowne (1892, p. 390) calls the portion of the alimentary tract "extending from the discal sclerites to the prepharyngeal tube, which may be regarded as its posterior limit or isthmus faucium" the *mouth* (L.). He (p. 141) points out that the whole of the haustellum, except the labrum-epipharynx and hypopharynx, together with the oral disc form a lower lip, which he calls the *pseudo-labium* (L.).



Text-fig. 3.

terminal, post-cochleariform portions of these folds numerous long hairs arise, which form *brushes*[1] (G. 2) (Text-fig. 3, *2*), overlying the anterior collecting channels (Text-fig. 2, IV, V). Centrally the brushes of the two sides meet and

[1] Suffolk (1869) figures the *brushes* (Pl. XIV), but does not name or describe them. Gleichen (1790) called the maxillary palps "brushes," but in this sense the term has long been disused.

roof over the distal portion of the dorsal seam. During the act of feeding the brushes not only prevent fluid from entering the distal portion of the dorsal seam, but in conjunction with the laterally placed tracts of fine hairs (Text-fig. 2, *18*) direct fluid drawn towards the anterior end of the disc on to the pseudotracheal surface.

The mechanism for roofing over the anterior portion of the prestomum and labial gutter is so efficient that this portion of the canal under all circumstances acts as a tube, transmitting the negative pressure created by the movements of the pharynx to the prestomum and food from the prestomum to the pharynx.

VI. THE INTERNAL ANATOMY OF THE ROSTRUM, EXCEPT THE MUSCULAR SYSTEM.

The most important chitinous structure in the rostrum is the *fulcrum* (L.), which serves for the attachment of several important muscles and contains the *pharynx*.

The *fulcrum* consists of a long, narrow, strong, *ventral* (L.) or *posterior* (P.) *plate*, which forms the posterior wall of the pharynx. The ventral plate is produced at its upper end into a pair of long, curved processes, the *proximal* (L.), *lateral* (P.) or *posterior* (Hewitt) *cornua*, and at its lower end into a pair of short, anteriorly directed processes, the *distal cornua* (L.). The ventral plate

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Text-fig. 3. Diagrammatic longitudinal section of the proboscis. *A*, *B*, *C*, lines along which the labella are folded back in the "filtering," "scraping" and "direct-feeding" positions respectively.

1. Inner surface of the labellum grooved by the pseudotracheae, with the lateral process of the furca and the epifurca, which are situated in its outer wall, showing through. *2*. Brush, covering the common anterior collecting channel. *3*. Cochleariform process of the discal sclerite. *4*. Superior disco-haustellar fold. *5*. Inferior disco-haustellar fold. *6*. Discal sclerite (stippled), bearing on its distal surface the prestomal teeth (black). *7*. Ventral wall of the labial gutter. *8*. Labrum-epipharynx (dotted). *9*. Section of hyoid sclerite. *10*. Anterior arch of fulcrum. *11*. Trapezoidal plate, attached to the anterior arch by its inferior syndesmosis. *12*. Dilator muscle of pharynx. *13*. Oesophagus. *14*. Pharynx, between the dorsal and ventral plates of the fulcrum. On the dorsal plate a series of minute, backwardly directed hairs is indicated. *15*. Common afferent salivary duct passing to the salivary pump.

The upward arrows I–VII indicate the planes of the transverse sections illustrated below. The downward arrow points to the illustration in each case. IIA represents the upper part of II enlarged.

a. Superior disco-haustellar folds interlocking and forming the "dorsal seam." *b*. Brush, overhanging the common anterior collecting channel. *c*. Outer wall of the labellum. *d*. Inner wall of the labellum grooved by the pseudotracheae. *e*. Lateral fold, in the outer wall of the labellum. *f*. Marginal strip. *g*. Inferior disco-haustellar folds (overlapping). *h*. Prestomal teeth arising from the discal sclerite. *i*. Lateral process of furca. *j*. Superior disco-haustellar folds separating and passing on to the surface of the haustellum. *k*. Hypoglossa, forming the ventral wall of the labial gutter. *l*. Paraphysis. *m*. Cavity of labial gutter. *n*. Inferior disco-haustellar folds separating. *o*. Mentum. *p*. Labrum-epipharynx (dotted). *q*. Hyoid sclerite. *r*. Efferent salivary duct. *s*. Lateral plate of fulcrum. *t*. Dorsal plate of fulcrum into which the dilator muscle of the pharynx (*x*) is inserted. (The hairs projecting into the cavity of the pharynx have been omitted.) *u*. Cavity of the pharynx between the dorsal and ventral plates of the fulcrum. *v*. Ventral plate of fulcrum. *w*. Common afferent salivary duct. *x*. Dilator muscle of pharynx. *y*. Median ridge of dorsal plate of fulcrum. *z*. Discal sclerite.

ends between the bases of the proximal cornua in a strong, concave edge, but between the distal cornua, though it seems to end similarly, the plate is continued as a very thin sheet of chitin, with a slightly strengthened edge, as far as the limits of the cornua.

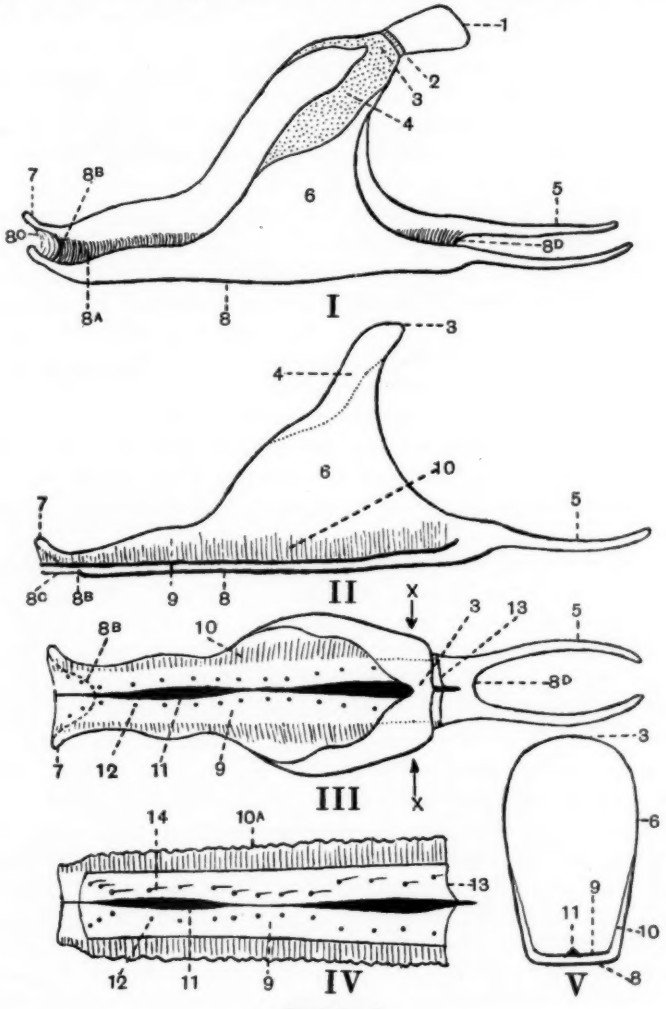
From the sides of the ventral plate arise triangular *lateral plates* (L.), which project forwards nearly at right angles to the ventral plate, except near their apices where they are thicker and bend inwards to meet and fuse forming the *anterior arch of the fulcrum* (P.). The anterior arch is situated on the surface of the rostrum immediately below the trapezoidal plate, to which it is united by the interior syndesmosis of the latter. In the region of the anterior arch the fulcrum is therefore quadrilateral in section. Distally the lateral plates of the fulcrum do not meet anteriorly, and the fulcrum is roofed over by thin integument.

Within the fulcrum a thinner plate of chitin, the *dorsal* or *epipharyngeal plate* (L.) of corresponding size to the ventral plate, lies above the latter. The dorsal plate extends distally beyond the strong edge of the ventral plate, and is strengthened by a *median ridge* or *raphé* (L.). On either side of this ridge "a row of fine setae project into the interior of the cavity, their points being turned towards the oesophagus; these are probably sensory organs by which the act of swallowing is controlled" (Lowne, 1870, p. 42). From the sides of the dorsal plate thin sheets of chitin pass forwards to fuse with the inner walls of the lateral plates. Close to the pharyngeal cavity the lateral wall of the fulcrum on each side consists therefore of two *lamellae*, the outer continued into the ventral plate and the inner into the dorsal plate. The *pharynx* is bounded, therefore, by the dorsal and ventral plates and the laminae of the lateral plates. The space between the lateral plates (anterior to the dorsal plate) is occupied by the intrinsic or dilator muscles of the pharynx which arise from the anterior arch and the adjacent integument and are inserted into the ridge and neighbouring parts of the dorsal plate. When these muscles contract the dorsal and ventral plates are drawn apart. The suction thus created draws the food up from the prestomum into the cavity of the pharynx.

Text-fig. 4. Diagrams representing the fulcrum after maceration. I. Lateral view, with dorsal plate removed (slightly tilted). II. Longitudinal section with dorsal plate in position. III. Anterior view with dorsal plate in position. IV. Dorsal plate (pharyngeal surface). V. Transverse section at line *x*...*x* in III.

1. Trapezoidal plate. *2*. Inferior syndesmosis of trapezoidal plate. *3*. Anterior arch. *4*. The portion of the lateral plate which is visible on the anterior surface of the rostrum below the anterior arch. *5*. Proximal cornu. *6*. Lateral plate. *7*. Distal cornu. *8*. Ventral plate. *8*A. Inner surface of ventral plate forming the floor of the pharynx. *8*B. Concave distal limit of the stronger portion of the ventral plate. In III this is represented as seen through the dorsal plate. *8*C. Thinner portion of ventral plate, distal to *8*B. *8*D. Concave proximal edge of ventral plate. *9*. Dorsal plate. *10*. Inner lamella, supporting dorsal plate and attached dorsally to the outer lamella, or lateral plate. *10*A. Cut margin of inner lamella. *11*. Median ridge of dorsal plate. *12*. Scars of setae on dorsal plate. *13*. Proximal limit of dorsal plate. *14*. Setae on dorsal plate, directed towards the oesophagus. (On the opposite side of the ridge, the scars (*12*) only are represented.)

The *hyoid sclerite* (L.) lies immediately distal to the fulcrum and surrounds the buccal cavity. It is a strong hoop of chitin, shaped like a signet-ring, narrow and incomplete anteriorly and broad posteriorly, so that when viewed from the side it appears to be triangular (Text-fig. 10, *K*). It is attached



Text-fig. 4.

by loose membrane to the dorsal and ventral plates of the pharynx proximally and to the labrum-epipharynx and hypopharynx distally.

In most descriptions of the proboscis little notice is taken of the hyoid sclerite, but though affording no attachment to muscles, and playing only a

passive part in the mechanism of feeding it has several important functions. It prevents the buccal cavity from collapsing either when suction is created by the pharynx or when the haustellum is flexed on the rostrum. Further, by establishing two closely approximated joints in the skeleton of the proboscis it permits of great flexion of the haustellum on the rostrum. Also it seems to prevent over-extension of the haustellum when the proboscis is fully erected, since the proximal posterior edge of the hyoid is then in contact with the distal end of the fulcrum, and its distal, posterior end engages in a transverse groove in the base of the hypopharynx. When the proboscis is strongly flexed the proximal end of the labrum-epipharynx and the distal end of the fulcrum lie within the cavity of the hyoid sclerite.

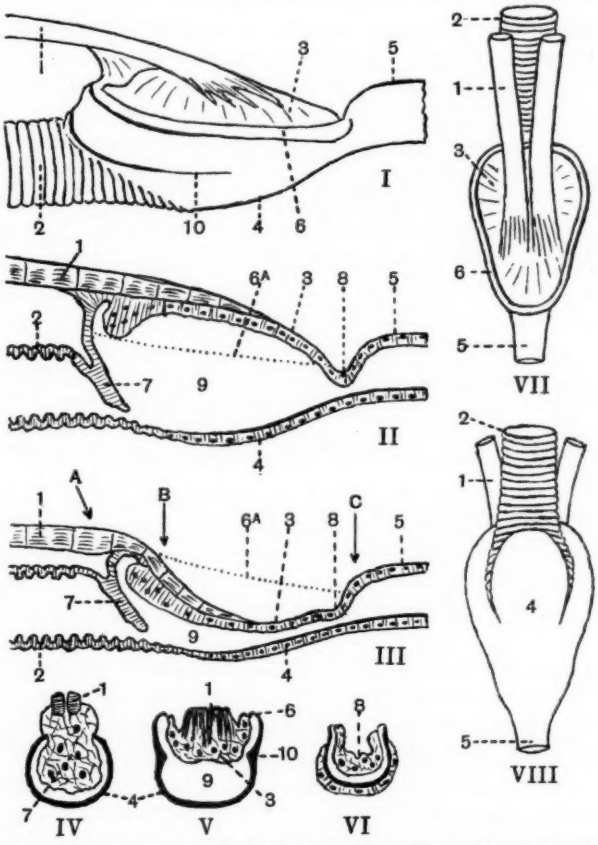
The *apodemes* (L.) of the labrum are two stout, slightly curved rods of heavily pigmented chitin, lying on each side of the fulcrum. Their upper ends, which are level with the centre of the fulcrum, are broad and flat for muscular attachment. In the upper three-quarters of their length they converge and then, after a pronounced *flexure* (G. 2), run parallel in a ventral direction to be inserted by their pointed extremities into small pits in the lateral cornua of the labrum, as it leaves the rostrum (Text-fig. 8).

Ventral to the fulcrum lie the *salivary ducts and pump*. The ducts of the two *lingual salivary glands* unite in the cervical region to form a *common afferent salivary duct*, strengthened by chitinous rings, which passes down behind the fulcrum as far as its lower third, where it enters the salivary pump.

The *salivary pump* is an oval cavity with thick chitinous posterior and lateral walls, the latter rising to a definite oval "rim." The anterior wall arising from the rim is thin and nucleated, and in the resting position hangs down into the cavity of the pump, almost touching the posterior wall near the origin of the efferent duct. The chitinous rings of the common afferent duct spread out on to the postero-lateral walls, but the rest of the pump and the efferent duct are without chitinous rings. The two *gracilis muscles*, arising from the posterior surface of the fulcrum near the bases of the posterior cornua, are attached to the anterior wall, proximally by tendinous insertions and distally by the fibres spreading fanwise over the surface of the anterior membranous wall. In longitudinal section a fold of the anterior wall seems to hang like a "clap valve" over the entrance of the afferent duct. The efferent duct conducts the saliva from the pump into the chitinous *salivary canal* of the hypopharyngeal sclerite of the haustellum.

Contraction of the gracilis muscles lifts the anterior wall out of the cavity of the pump and by the suction thus created draws saliva from the afferent duct into the pump. When the muscles relax the anterior wall returns into the cavity forcing the saliva, which is prevented by the "clap valve" from re-entering the afferent duct, into the efferent duct and eventually into the labial gutter. The downward fold of the anterior wall, which almost closes the commencement of the efferent duct, perhaps acts as a valve to prevent suction

of air into the pump when the gracilis muscles first contract, and backward flow of saliva from the efferent duct into the pump subsequently[1].



Text-fig. 5. The salivary pump. I. Lateral view with the gracilis muscles contracted and raising the anterior wall. II. Longitudinal section, under the same conditions. III. Longitudinal section with the muscles relaxed. IV, V, VI. Transverse sections at *A*, *B* and *C* in III. VII. Anterior view. VIII. Posterior view.

1. Gracilis muscle, spreading, at its insertion, over the anterior wall. *2.* Common afferent duct, with chitinous rings. *3.* Nucleated anterior wall of pump. *4.* Thick chitinous posterior wall. *5.* Efferent duct, without chitinous rings. *6.* Thickened "rim" of lateral wall. 6^A. Position of "rim." *7.* "Clap valve" of afferent duct. *8.* Fold closing the entrance to the efferent duct. *9.* Cavity of pump. *10.* Strengthening ridge of chitin in the lateral wall.

[1] Lowne (1870, p. 52) speaks of it "as a most perfect valve; for its superior wall is elastic and pressed down at its anterior portion against the inferior wall, so entirely closing the duct at this point."

Cornwall (1923) gives the following description of the salivary pump of *Stomoxys calcitrans*. "The common afferent duct is a tube stiffened with chitinous rings which spread out over the posterior two-thirds of the convexity of the dilatation which forms the pump cavity. A membrane

Apart from the muscles the only other structures in the rostrum are the *nerves* and *air sacs*.

The two *labial nerves* (L.) run down behind the fulcrum on each side of the salivary duct in front of the retractor muscles of the fulcrum, giving off twigs to the various muscles and larger branches to the palps.

Lowne (p. 396) states that a pair of large *air sacs* derived from the cervical tracheae enter the rostrum, where each divides into two branches, an anterior, "which lies on the outer side of the fulcrum and a posterior which descends behind the fulcrum." He states that each trunk before it leaves the neck "has a slender rod of chitin in its anterior wall, which closes the tube by pressing against the jugum. This forms a valve capable of being opened by a small bundle of muscle fibres, which arise from the front edge of the gena; their contraction opens the valve and permits of the passage of air from the thoracic cavity into the tracheae of the proboscis."

Hewitt (1914, p. 45) says that in the house-fly there is on each side of the rostrum a single, unbranched sac, continuous with the ventro-lateral cephalic sac, lying posterior to the fulcrum.

VII. The internal anatomy of the haustellum.

The *labium* (P.) or *theca* (L.)[1] forms the greater part of the haustellum. Its wall, which is a continuation of the wall of the rostrum, consists of loose membrane, thickened on the anterior and posterior aspects into definite plates.

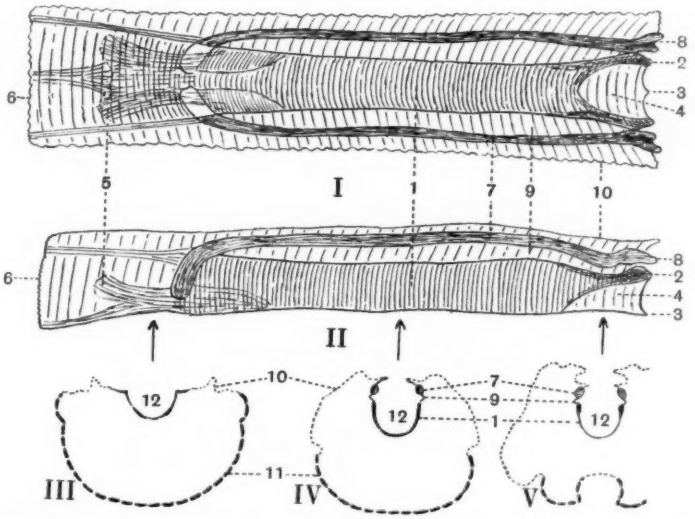
The plate on the posterior aspect, known as the *mentum* or *thyroid*, has been described. Distally it articulates, through the mento-furcal bars, with the furca (see p. 81).

The plate on the anterior surface forms the walls of the longitudinal depression in the labium called the *labial gutter*. The central portion of this plate, forming the floor of the gutter, is thick, especially in its distal two-thirds, concave in its transverse diameter, and extends along the whole length of the gutter. Its upper third is thinner, though its edges and central area are moderately strong, and proximally it fuses with the hypopharynx. Distally the central plate ends in a well-defined, notched edge, with a central *spine* (G. 2), and two thickened *lateral cornua* (G. 2). In a semicircular area between the lateral cornua the chitin is thin. This plate is called by Lowne the *hypoglossa* (L.) or *stomal plate* (L.). From the outer sides of this plate, at the junction of its proximal and middle thirds, arise two strong bars of chitin, the *paraphyses* (L.), which lie parallel with its distal portion. These bars terminate in strong, expanded, notched, articular *heads* (G. 2), a little beyond

containing nuclei falls into the cavity to which two or more muscles are attached. Contraction of these muscles pulls out the membrane and into the space thus formed saliva flows from the afferent duct. When the muscles relax the membrane slaps back by virtue of the elasticity of its attachments, covers as a valve the opening of the afferent duct and forces the fluid in the pump cavity along the efferent duct to the proboscis. The efferent duct has no rings, but thick, double walls...similar arrangements are found in non-blood-sucking *Muscidae*."

[1] Hewitt (1914, p. 14) restricts the name *theca* to the plate of chitin here called the mentum.

the level of the distal end of the hypoglossa, and from their anterior margins folds of chitin, already described (p. 57) as the inferior disco-haustellar folds, project over the gutter. The paraphyses are connected throughout their whole length to the hypoglossa by thinner chitin, which is especially loose distally and allows of considerable alterations between the relative positions of the distal extremities of the hypoglossa and the paraphyses. Distally the



Text-fig. 6. The hypoglossa. I. Anterior view (flattened). II. Lateral view, after removing the surrounding parts of the haustellum. III, IV, V. Sections through the haustellum to show the relationship of the hypoglossa to the rest of the integument. The parts of the integument not shown in I and II are dotted.

1. Central plate of hypoglossa, forming the floor and sides of the labial gutter. *2.* Thickened *lateral cornua* of the hypoglossa, which articulate with the discal sclerite. *3. Spine. 4.* Thin semicircular area between the lateral cornua. *5.* Thickened portion in the proximal ventral wall of the gutter. *6.* Line of fusion of hypoglossa with hypopharynx. *7.* Paraphysis. *8.* Distal notched head of paraphysis. *9.* Thin membrane which connects the central plate of the hypoglossa to the paraphysis. *10.* Membrane forming the outer wall of the haustellum. (It is thickened dorsally to form the hypoglossa and ventrally to form the mentum (*11*).) *12.* Cavity of labial gutter.

hypoglossa and the paraphyses articulate with each other and also with the discal sclerite in a manner to be described later (p. 79).

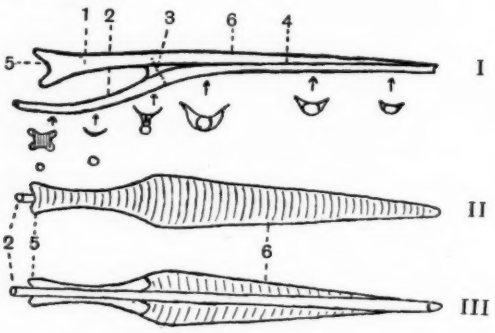
In the different feeding positions the relative positions of the anterior and posterior plates of the labium, the mentum and hypoglossa, are altered to some extent, the former moving distally in relation to the latter as the labella are rotated backwards.

The *labial nerve* continues down from the rostrum, lying between the muscles, which occupy the interior of the labium.

The posterior air sac "on reaching the haustellum takes the form of a *trachea* proper, having annular thickenings" as it does in the house-fly (Hewitt, 1914), and accompanies the labial nerve sending off twigs to the muscles.

The other structures in the haustellum are the hypopharynx and the labrum-epipharynx, both of which lie anterior to the labium in the labial gutter.

The *hypopharynx* or *ligula* (L.) (Text-fig. 7) fulfils two main functions, namely as a chitinous canal for the terminal part of the efferent salivary duct and, by its articulation with the epipharynx, as a ventral wall for the alimentary canal at and beyond the part called by Lowne the prepharyngeal tube. The portions fulfilling these functions are distinct, though united in the distal two-thirds of the organ.



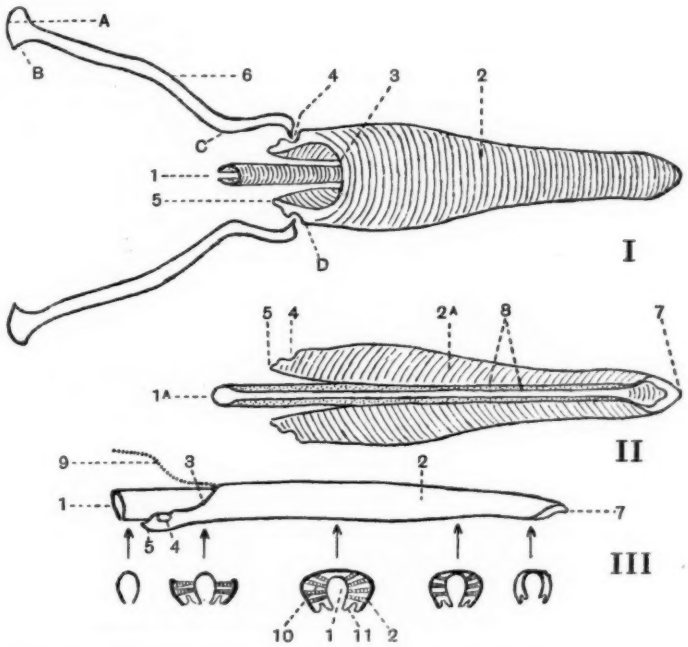
Text-fig. 7. The hypopharynx. I. Side view, with transverse sections, at the points indicated by the arrows below. II. Anterior surface. III. Posterior surface.

1. Free portion of dorsal plate (handle). *2.* Free portion of salivary canal (guard). *3.* Line of fusion with hypoglossa. *4.* Fused dorsal plate and salivary duct (blade). *5.* Proximal end of dorsal plate with notched articular surface. *6.* Edge of dorsal plate, which articulates with the labrum-epipharynx.

The hypopharynx lies mainly in the labial gutter, with the extreme proximal end of whose ventral wall, the hypoglossa, it is fused. The smaller portion of the hypopharynx proximal to the fusion, later called the handle, may be considered to project into the rostrum.

The hypopharynx is dagger-shaped, the proximal third representing the *handle* (G. 2) with a guard and the distal portion the *blade* (G. 2). The *salivary canal* (G. 2) or tubular part, pierced for the salivary duct, which lies ventrally throughout, is fused to the dorsal plate (blade) in the distal two-thirds of the organ in the manner shown in Text-fig. 7, but in the proximal third, where it carries the duct over the haustello-rostral joint, becomes free and curved, looking like a guard to the handle. The *dorsal plate* (G. 2) is concave in its transverse diameter throughout its length, and articulates by its edges with the membrane between the free edges of the labrum and epipharynx. It is narrow distally, but gradually widens to the point where it becomes free from the

salivary canal, and then again narrows slightly, forming the "handle" of the dagger. Its proximal end is composed of strong, highly pigmented chitin, deeply notched both transversely and vertically, so that it terminates in four small cornua. The transverse notch articulates with the hyoid and the posterior



Text-fig. 8. The labrum-epipharynx. I. Dorsal view. II. Ventral view (apodemes omitted). III. Lateral view (apodemes omitted). Transverse sections at the points indicated by the arrows are shown below.

1. Epipharynx. *1A*. Ventral surface of epipharynx, showing the longitudinal slit. *2*. Labrum. *2A*. Ventral surface of the labrum, showing the longitudinal slit, through which the epipharynx is visible. *3*. Semicircular proximal (dorso-lateral) margin of the labrum to which the membranous wall of the rostrum (*9*) is attached. *4*. Pit for the articulation of the apodeme. *5*. Lateral cornu of labrum. *6*. Apodeme. *7*. Delicate distal extremity of labrum. *8*. Ventral edges of labrum and epipharynx which are united by a thin membrane (*11* in section), indented to receive the lateral edge of the hypopharynx. *10*. Dilator muscle of the labrum-epipharynx.

A. Insertion of the extensor muscle of the haustellum. *B*. Insertion of the adductor muscle of the apodeme. *C*. Insertion of the flexor muscle of the haustellum. *D*. Insertion of the flexor muscle of the labrum.

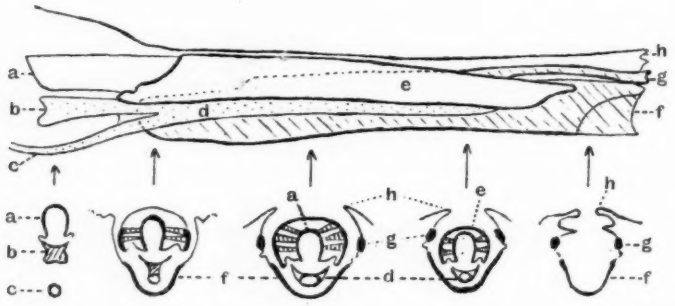
portion of the membranous wall of the buccal cavity is fused to the posterior edge of the notch. The line of fusion with the hypoglossa embraces the organ, except the anterior surface of the dorsal plate, immediately distal to the commencement of the handle.

The saliva is discharged from the distal end of the salivary canal into the labial gutter near its distal end.

The relation of the hypopharynx to the hypoglossa is shown in Text-fig. 9.

The *labrum-epipharynx* (L.) lies for the greater part of its length in the labial gutter dorsal to the hypopharynx, with which it articulates.

It consists of two tubular sheets of thick, pigmented chitin, each incomplete ventrally. The ventral plate, the *epipharynx*, is enclosed within and at its distal end *fused* with the dorsal, the *labrum*, and two stout rods, the *apodemes* (see p. 62), articulate with the latter. When viewed from the dorsal surface (Text-fig. 8) the labrum-epipharynx looks like a blunt, barbed and tanged arrow-head, the blade being formed by the body, the barbs by the lateral cornua of the labrum and the tang by the ventrally projecting, proximal end of the epipharynx.



Text-fig. 9. Diagram to show the relationship of the labial gutter (*f*, diagonal lines) to the hypopharynx (*d*, stippled) and the labrum-epipharynx (*e*). The labial gutter is represented in longitudinal section. Below are shown transverse sections at the points indicated by the arrows.

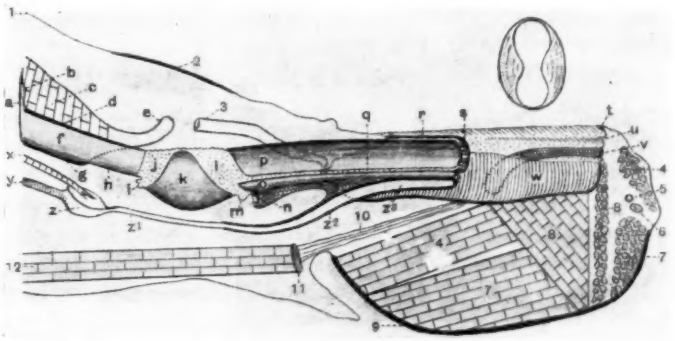
a. Epipharynx. *b*. Dorsal plate of hypopharynx. *c*. Salivary canal. *d*. Blade of hypopharynx after the union of the dorsal plate and salivary canal. *e*. Labrum-epipharynx. *f*. Hypoglossa. *g*. Paraphysis. *h*. Disco-haustellar fold.

Except its distal extremity, which is delicate and flattened and has on its ventral surface sensory tubercles, the *labrum* is composed of a strong sheet of deeply pigmented, smooth chitin in the form of a long cone, open at both ends and also ventrally. Proximally it ends in a smooth, semicircular margin, with its concavity towards the rostrum, to the anterior membranous wall of which it is fused. The ends of the semicircular margin are produced on each side of the ventral gap into strong *lateral cornua* (G. 2), provided with pits on their outer surfaces into which the pointed, distal ends of the apodemes are received.

The *epipharynx* is also composed of a sheet of chitin in the form of a tube, open at both ends and also ventrally, and, except its free proximal fourth, lies within the labrum, with which it is fused at its distal end dorsally.

Narrow strips of thin membrane, grooved longitudinally for articulation with the dorsal plate of the hypopharynx, unite the ventral free edges of the labrum and epipharynx (Text-fig. 8, Sections).

Short fan-like muscles, the *dilators of the labrum-epipharynx*, arising from the internal surface of the labrum and inserted into the outer surface of the epipharynx, are presumably capable of altering the diameter of the cavity of the epipharynx.



Text-fig. 10. Diagrammatic median longitudinal section of the lower third of the rostrum and upper third of the haustellum.

a. Lateral plate of fulcrum. *b*. Inner lamella of lateral plate of fulcrum. *c*. Dilator muscle of pharynx. *d*. Dorsal plate of fulcrum. *e*. Distal cornu of fulcrum. *f*. Cavity of pharynx. *g*. Ventral plate of fulcrum. *h*. Thin portion of ventral plate. *i* and *j*. Loose membrane connecting the plates of the fulcrum with the hyoid sclerite. *k*. Hyoid sclerite. *l* and *m*. Loose membrane connecting the hyoid sclerite with the hypopharynx and epipharynx. *n*. Proximal portion of the dorsal plate of the hypopharynx. *o*. Edge of hypopharynx articulating with the labrum-epipharynx. *p*. Epipharynx. *q*. Membrane connecting the free edges of the labrum and epipharynx, and grooved for the reception of the edge of the dorsal plate of the hypopharynx. *r*. Labrum. *s*. Transverse section of labrum-epipharynx with dilator muscles (cut off at this point). *t* and *u*. Superior and inferior disco-haustellar folds respectively. *v*. Paraphysis. *w*. Hypoglossa, forming ventral and lateral wall of labial gutter. *x*. Gracilis muscle. *y*. Afferent salivary duct. *z*. Salivary pump. z^1. Efferent salivary duct. z^2. Free portion of salivary canal. z^3. Portion of salivary canal fused to the dorsal plate of the hypopharynx.

1. Anterior wall of rostrum. *2*. Section of palpigerous scale. *3*. Apodeme. *4*. Retractor of paraphysis. *5*. Trachea. *6*. Labial nerve. *7*. Retractor of furca. *8*. Transverse muscle of haustellum. *9*. Mentum. *10*. Fibrils connecting sesamoid sclerite and hypoglossa. *11*. Sesamoid sclerite. *12*. Retractor of rostrum.

Inset. Anterior view of the hyoid sclerite.

The distal end of the labrum-epipharynx almost reaches to the discal sclerite.

An attempt has been made in Text-figs. 9 and 10 to illustrate relationships of the labrum-epipharynx and the hypopharynx to each other and to the labial gutter.

VIII. The muscles of the proboscis.

Since only one muscle, the retractor of the furca, passes beyond the haustellum it seems most convenient to consider the musculature of the proboscis before describing the anatomy of the oral disc.

The muscles have been carefully dissected and compared with the descriptions given by Lowne for *C. erythrocephala* and by Hewitt (1914) for *M. domestica*. When the nomenclature of these two workers differs both names are quoted, Lowne's name usually first, as claiming priority and being generally the most suitable. Three new names have been suggested and a new muscle described.

(1) *Muscles arising in the head capsule.*

The retractors of the fulcrum (L. and Hewitt) arise from the internal anterior edges of the genae and are inserted into the extremities of the posterior cornua of the fulcrum. "Their contraction causes the lower end of the fulcrum to describe a circular arc, and brings it into a horizontal position between the genae" (Lowne). (See Text-fig. 13, III.)

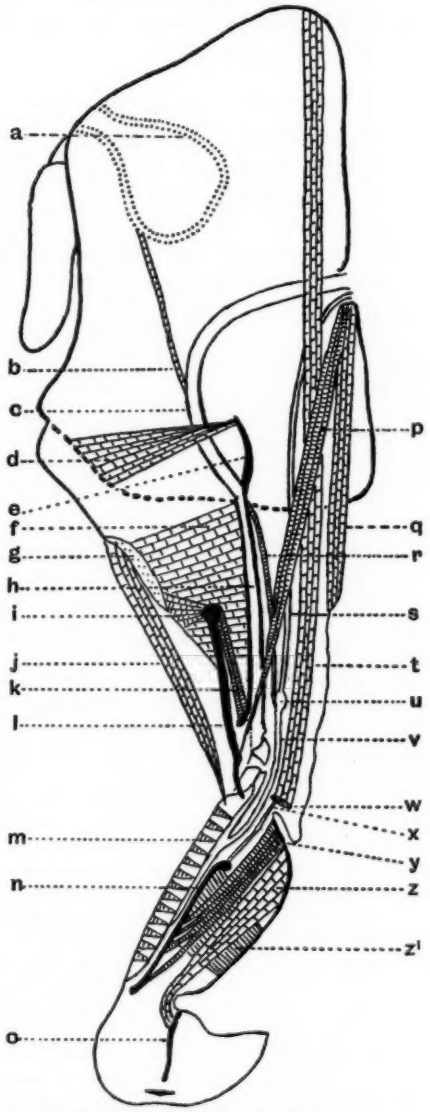
The *retractors of the rostrum* (L.) [= *retractors of haustellum*, Hewitt, p. 59] arise from the dorso-lateral regions of the occiput. "They are long and slender. They lie on either side of the middle line behind the lingual salivary duct; they are inserted into the sesamoid sclerites" (Lowne). From the sesamoid sclerites tendon-like fibrils seem to pass to the slightly thickened area on the ventral surface of the hypoglossa near the proximal ends of the paraphyses. (Text-fig. 10, *10*.) A few seem to be inserted into the distal extremity of the mentum.

The *accessory retractors of the rostrum* (L.) [= *retractors of the rostrum*, Hewitt] arise near the last at the sides of the occipital foramen and pass downwards with them to be inserted into the integument on the ventral side of the rostrum "midway between the sesamoid sclerites and the head capsule" (Lowne).

"The last two pairs of muscles acting together assist in the retraction of the whole proboscis" (Hewitt). (See Text-fig. 13, III.)

The *flexors of the haustellum* (L. and Hewitt) arise from the sides of the occipital foramen and are inserted into the flexures of the apodemes of the labrum. They "serve to flex the haustellum on the anterior face of the rostrum" (Hewitt). (Lowne stated that they were "inserted into the dorsal surface of the prepharyngeal tube.")

The *retractors of the oesophagus* (G. 2) "are small bundles of muscle fibres which arise from the lower part of the frontal sac, or remains of the ptilinum, and which are inserted into the muscular coat of the oesophagus between the fulcrum and the brain.... They serve to draw the loop of oesophagus, which lies between the cephalic ganglia and the fulcrum, forward, when the proboscis is retracted into the head capsule" (Lowne, p. 392). Lowne terms these muscles *retractors of the fulcrum*. In free-hand longitudinal sections of the



Text-fig. 11. Diagram showing the muscles of the proboscis.

a. Remains of the ptilinal sac. *b*. Retractor of the oesophagus. *c*. Oesophagus. *d*. Retractor of fulcrum. *e*. Posterior cornu of fulcrum. *f*. Dilator of pharynx. *g*. Anterior arch. *h*. Cavity of pharynx. *i*. Adductor of apodeme. *j*. Flexor of labrum. *k*. Extensor of haustellum. *l*. Apodeme. *m*. Dilator of labrum-epipharynx. *n*. Paraphysis. *o*. Lateral process of furca. *p*. Flexor of haustellum. *q*. Accessory retractor of rostrum. *r*. Gracilis. *s*. Afferent salivary duct. *t*. Retractor of rostrum. *u*. Salivary pump. *v*. Efferent salivary duct. *w*. Sesamoid sclerite. *x*. Fibrils passing from sesamoid sclerite to hypoglossa. *y*. Retractor of paraphysis. *z*. Retractor of furca. z^1. Transverse of haustellum.

head these fibres are often visible, but they are not mentioned by Kraepelin, Hewitt or Patton and Cragg.

(2) *Muscles arising in the rostrum.*

The *extensors of the haustellum* (L. and Hewitt) arise from the distal cornua and neighbouring parts of the fulcrum and are inserted into the *outer* sides of the heads of the apodemes. They extend the haustellum on the rostrum by pushing down the apodemes.

The *adductors of the apodemes* (G. 2) [= *retractors of the haustellum*, L.; *accessory flexors of the haustellum*, Hewitt] arise from the anterior distal margins of the lateral plates of the fulcrum and are inserted near the extensors of the haustellum into the *inner* sides of the heads of the apodemes. In the resting position the apodemes diverge, but when the haustellum is extended become almost parallel. These muscles seem to assist the extensors of the haustellum by adducting the heads of the apodemes.

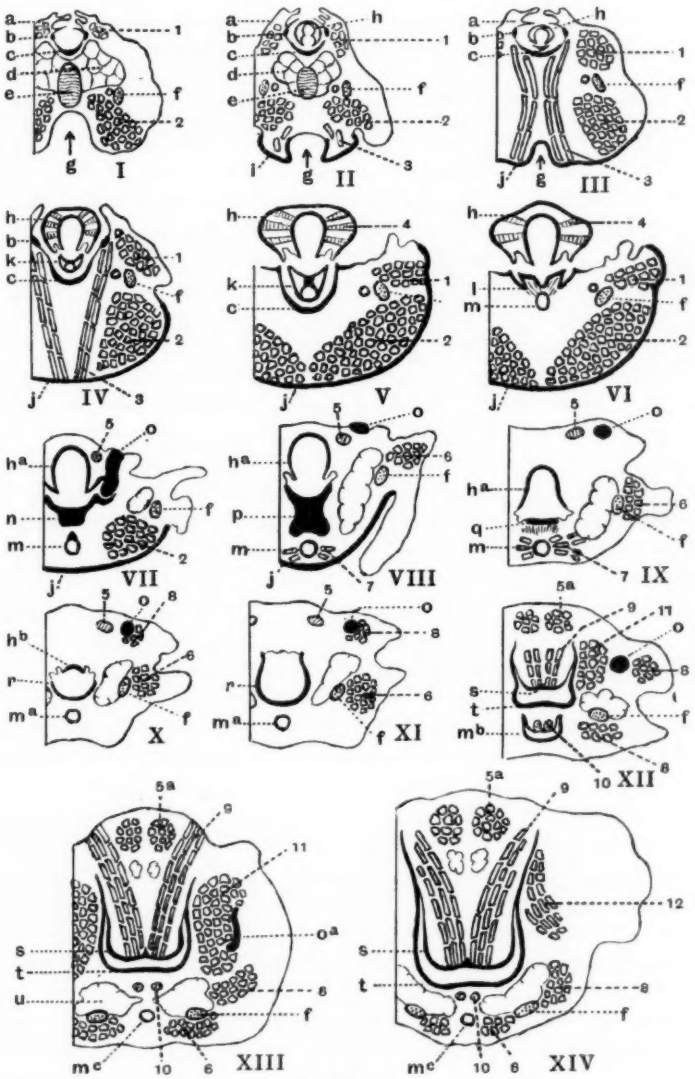
Lowne agrees with Kraepelin that these and the extensors of the haustellum act together and by pushing down the apodemes straighten the proboscis by extending the haustellum on the rostrum. Hewitt on the other hand thinks they "assist in the retraction of the proboscis by flexing the haustellum on the rostrum."

Text-fig. 12. Transverse sections through the haustellum and distal half of the rostrum. I. Just proximal to discal sclerite. II. Through forks of the mentum. III. Through the mentum, close to the origins of the forks. IV. Near Text-fig. 3, IV. V. Near Text-fig. 3, V. VI. Through the point of fusion of the hypoglossa and hypopharynx. VII. Through the articulation of the apodeme with the labrum. VIII. Through the "handle" of the hypopharynx. IX. Slightly proximal to VIII. X. Through the distal portion of the hyoid sclerite. XI. Through the centre of the hyoid sclerite. XII. Through the salivary pump and distal portion of the fulcrum. XIII. Through the central portion of the fulcrum. XIV. Through the fulcrum just distal to the anterior arch.

a. Disco-haustellar folds (see Text-fig. 3). *b*. Paraphysis. *c*. Hypoglossa. *d*. Labial salivary gland. *e*. Elastic ligament. *f*. Labial nerve, accompanied internally by a tracheal trunk in I to VI and by a tracheal sac (*u*) in VII to XIV. *g*. Groove on posterior surface continued proximally between the forks in II and in the mentum in III. *h*. Labrum-epipharynx. h^a. Epipharynx. *i*. Fork of mentum. *j*. Mentum. *k*. Hypopharynx ("blade"), including the salivary canal. *l*. Fusion of hypopharynx and hypoglossa. *m*. Free portion of salivary canal. m^a. Efferent salivary duct. m^b. Salivary pump. m^c. Afferent salivary duct. *n*. Distal portion of the "handle" of the hypopharynx. *o*. Apodeme. (In VII it articulates with the labrum-epipharynx, and in X and XI the insertion into it of the flexor of the haustellum is seen.) o^a. Expanded head of the apodeme. *p*. Quadrilateral part of the "handle" of the hypopharynx. *q*. Extreme proximal end of the posterior margin of the transverse cleft in the "handle" of the hypopharynx. *r*. Hyoid sclerite. (In X the extreme tip of the epipharynx h^b is seen within the distal portion of the hyoid.) *s*. Dorsal plate and *t* ventral plate of fulcrum, with the cavity of the pharynx between them. (In XII, XIII and XIV the lamellae of the lateral plates are seen.) *u*. Air sac lying internal to the labial nerve. (Other air sacs in the rostrum are omitted.)

Muscles. *1*. Retractor of paraphysis. *2*. Retractor of furca. *3*. Transverse of haustellum. *4*. Dilator of labrum-epipharynx. *5*. Tendon of flexor of labrum. 5^a. Flexor of labrum. *6*. Retractor of rostrum. *7*. Epipharyngeal, passing from the free portion of the salivary canal to the neighbouring air sac and the integument. *8*. Flexor of haustellum. *9*. Dilator of pharynx. *10*. Gracilis. *11*. Extensor of haustellum. *12*. Adductor of apodeme.

The *flexors of the labrum* (L. and Hewitt) arise from the distal edge of the anterior arch and from the adjacent margins of the lateral plates of the fulcrum



Text-fig. 12.

and are inserted by fine tendons into the sides of the lateral cornua of the labrum close to the articulations of the apodemes (Text-fig. 8, D). These muscles flex the haustellum on the rostrum.

The *gracilis muscles* (L.), which regulate the flow of saliva, arise from the posterior wall of the fulcrum near the bases of the posterior cornua and are inserted into the anterior wall of the salivary pump (p. 62).

The *dilators of the pharynx* (L.) arise from the internal surfaces of the anterior arch and lateral plates of the fulcrum and are inserted into the dorsal plate of the pharynx. They are the chief agents in creating the suction which draws food into the proboscis (see also p. 60).

The *epipharyngeal muscle* (G. 2) consists of fibres arising from the "handle" of the hypopharynx and the free portion of the salivary canal and passing to the neighbouring walls of the air sacs of the rostrum and to the adjacent integument. This small muscle, which is not mentioned by Lowne or Hewitt, probably assists in the disposition of the parts during flexion and extension. (Text-fig. 12, IX, 7.)

(3) *Muscles arising in the haustellum.*

The *retractors of the furca* (L. and Hewitt) arise from the proximal halves of the inner lateral surfaces of the mentum and are inserted into the tubercles of the lateral processes of the furca.

Contraction of these muscles causes the lateral processes, carrying the labella with them, to rotate outwards on the mento-furcal bars. Further contraction causes progressive folding back of the labella, accompanied by traction exerted through the tendinous cords, which spring from the epifurcas, on the proximal portions of the pseudotracheal membranes, first exposing and later everting the prestomal teeth. In fact the filtering, scraping and direct feeding positions are brought about by varying degrees of action of these muscles (see Text-fig. 35).

The *retractors of the paraphyses* (G. 2) arise from the lateral edges of the proximal half of the mentum, in front of the retractors of the furca, and are inserted by several slips into the distal thirds of the paraphyses and by tendons into their expanded heads. The action of this muscle is discussed later (p. 79).

These muscles seem to be the *retractors of the discal sclerites* of Hewitt, who states that they are inserted into the discal sclerite, and they may be the *paraphysal muscles* of Lowne.

The *transverse muscles of the haustellum* (L.) [= *dilator of the labium-hypopharynx*, Hewitt] are several muscular slips which arise from the internal surface of the mentum near the middle line, between the centre and the distal end, and run forwards and upwards to be inserted into the paraphyses. Lowne thinks "the real function" of these muscles "is to approximate the paraphyses," while Hewitt (p. 61), owing to a mistaken view as to their insertion, is of opinion that "by their contraction they will widen the channel of the labium-hypopharynx." Their action is discussed on p. 80.

The *dilators of the labrum-epipharynx* [= *prelabral*, L.] are short fan-like muscles, arising from the internal surface of the labrum and inserted into the

outer surface of the epipharynx, which presumably are capable of altering the diameter of the cavity of the epipharynx.

IX. Retraction of the proboscis into the head capsule.

The retraction of the proboscis into the head capsule is due entirely to muscular action. The retractors of the fulcrum cause this structure to rotate at the inferior syndesmosis of the trapezoidal plate until the long axis of the rostrum is nearly parallel with the lateral margin of the epistomal orifice. Simultaneously the flexors of the haustellum and labrum flex the haustellum on the rostrum. Finally the retractors and accessory retractors of the rostrum lift the whole proboscis within the head capsule. This movement is accompanied by rotation of the trapezoidal plate on its superior syndesmosis and probably by partial relaxation of the retractors of the fulcrum so that the upper end of the fulcrum is caused to lie immediately behind the facial plate (Text-fig. 13, III).

While the proboscis is retracted within the head capsule the oral disc can be moved into various positions, including maximum extension, and the labella can be separated and food regurgitated.

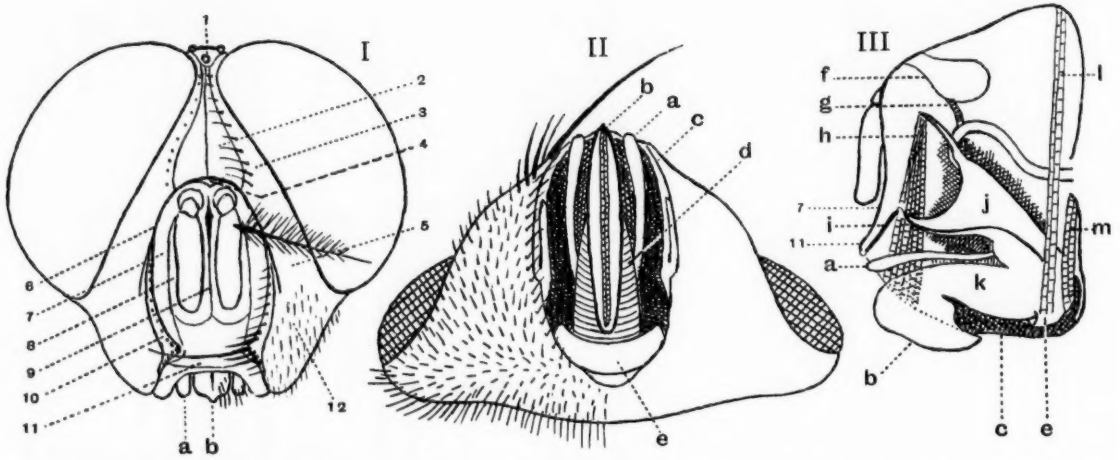
X. The oral disc.

The *oral disc* consists of *two lobes* (L.) or *labella* (P.) joined together behind by the *interlabellar membrane*, but with free anterior and inferior margins. The external features of the disc have been described, but in order to understand its functions it is necessary to give a detailed account of the structure of its various parts.

Each *labellum* presents two surfaces differing greatly from each other, the inner, known as the *pseudotracheal membrane* (P.), arising from the discal sclerite supports the prestomal teeth and is grooved by the pseudotracheae, and the outer, which joins the inner at the inferior or prestomal margin, supports numerous sensory hairs and passes proximally to fuse with the posterior and lateral walls of the haustellum. The outer walls are strengthened by the thick rods, known as the lateral processes of the furca.

The oral disc articulates on each side with the haustellum at two joints, one anterior between the discal sclerite and the hypoglossa and paraphyses, forming the distal end of the labial gutter, and one posterior between the furca and the forks of the mentum, through the intervening mento-furcal bars. In extreme flexion the posterior wall of the disc is applied to the ventral wall of the haustellum and lies in the depression between the forks of the mentum. Extension beyond the point at which the long axis of the disc is at right angles to the long axis of the haustellum is rendered impossible by the relation of the paraphyses to the discal sclerite.

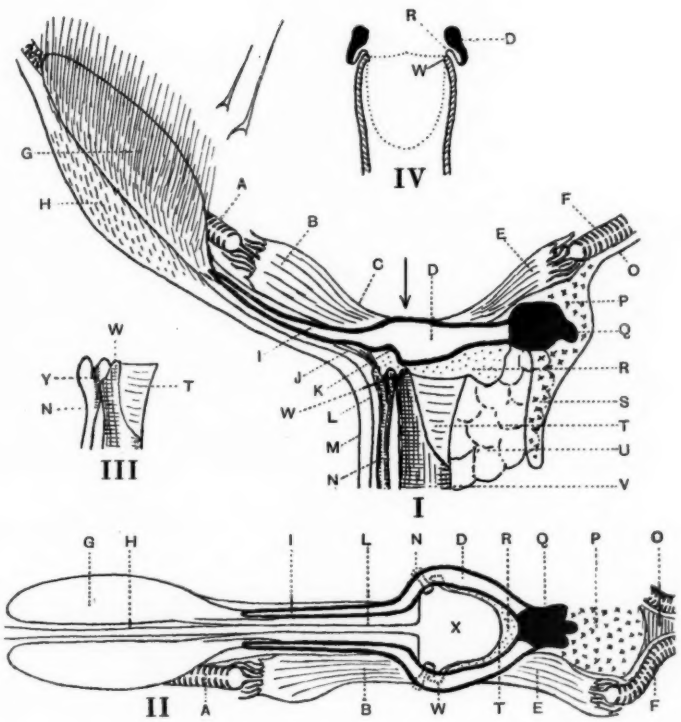
The *discal sclerite* (L.) consists of two strong, curved *bars* (G. 2) of chitin, with thinner lateral extensions, joined together posteriorly by a densely pigmented quadrilateral mass with a pointed extremity, the *nodulus* (L.). *Viewed from the side* the bar, which is about half the thickness of the nodulus,



Text-fig. 13. I. Front view of head of male, with proboscis almost completely retracted. II. Under side of head, with proboscis retracted. III. Longitudinal section, showing the position of the retracted proboscis.

1. Ocellar plate. *2*. Frontal plate. *3*. Parafrontal plate. *4*. Lunula. *5*. Parafacial (geno-vertical) plate. *6*. Ptilinal suture, enclosing within its arms the ptilinal area, facial depression or front. *7*. Meso-facial plate, facial plate or face. *8*. Antennal groove or fossa. *9*. Facial carina (a ridge between the antennae). *10*. Vibrissal or facial ridge. *11*. Epistome. *12*. Cheek or gena.

a. Maxillary palp. *b*. Oral disc (see Text-fig. 2, II). *c*. Margin of epistomal orifice. *d*. Mentum. *e*. Fold of integument covering, in the retracted position, the proximal end of the mentum. *f*. Remains of the ptilinal sac. *g*. Retractor of oesophagus. *h*. Retractor of fulcrum. *i*. Trapezoidal plate, flexed both on the epistome and on the fulcrum. *j*. Fulcrum. *k*. Haustellum. *l*. Retractor of rostrum. *m*. Accessory retractor of rostrum.



Text-fig. 14. The discal sclerite. I. Side view of inner surface, obtained by making a longitudinal section through the nodulus and the tissue posterior to it. The sclerite has been drawn away, as far as possible, from the paraphysis and hypoglossa. II. Dorsal view, after removing the labella. III. Diagram to show the groove in the paraphysis in which the distal cornu of the hypoglossa slides. IV. Diagrammatic section in the direction of the arrow in I, to show the articulation of the distal cornua of the hypoglossa with the bars of the discal sclerite.

A. Common anterior collecting channel covered by the "brush," *G*. *B*. Anterior lateral plate of the discal sclerite. *C*. Concave margin to which the prestomal teeth are articulated. *D*. Bar of the discal sclerite. *E*. Posterior lateral plate of the discal sclerite. *F*. Common posterior collecting channel. *G*. Brush. *H*. Hair tract. *I*. Cochleariform process. *J*. Lateral ligament, connecting the head of the paraphysis with the base of the cochleariform process. *K*. Paraphysal notch in bar of discal sclerite. *L*. Inferior disco-haustellar fold. *M*. Superior disco-haustellar fold. *N*. Paraphysis. *O*. Interlabellar membrane. *P*. Lateral portion of elastic ligament. *Q*. Nodulus. *R*. Thin membrane joining the paraphyses and hypoglossa to the discal sclerite, except between the paraphyses, where it is lacking. Posteriorly it is loose and forms a pouch, termed by Lowne (p. 144) the poculum. *S*. Process of elastic ligament. *T*. Thin semicircular area between the distal cornua of the hypoglossa. *U*. Labial salivary gland. *V*. Hypoglossa. *W*. Distal cornu of hypoglossa which articulates both with the bar of the discal sclerite (IV) and with the paraphysis (III). *X*. Oral aperture. *Y*. Groove in the head of the paraphysis in which the distal cornu of the hypoglossa slides.

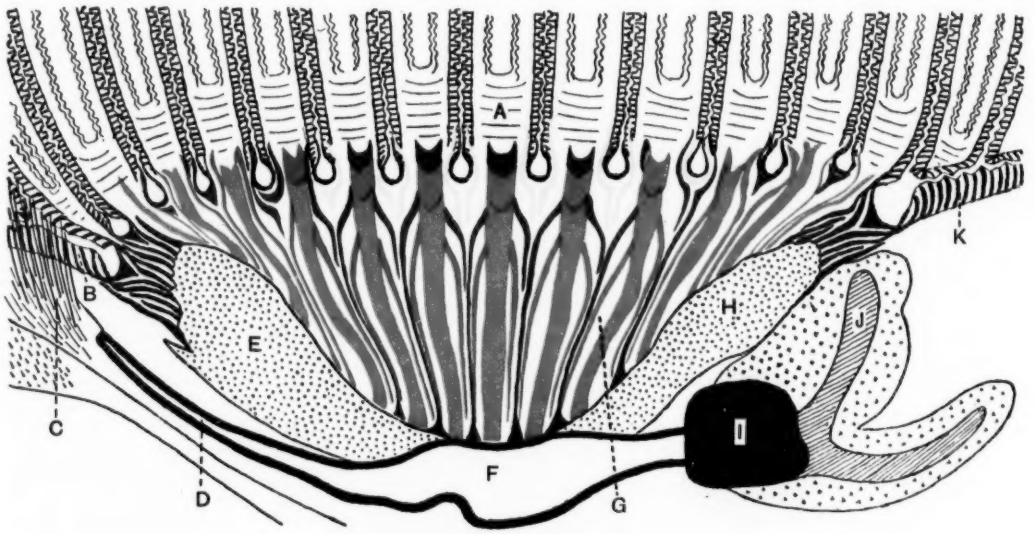
runs forward from it for a short distance and then expands. A distinct notch is present on the haustellar side of the expansion with which the head of the paraphysis articulates. Beyond the *paraphysal notch* (G. 2) the bar becomes thinner and curves distally to end in a pointed extremity. This narrow, curved portion is known as the *cochleariform process* (L.). A thin sheet of chitin, the *posterior lateral plate of the discal sclerite* (G. 2), springs from the outer side of the bar between the nodulus and the central expansion and supports the posterior part of the inner wall of the labellum, and a similar sheet, the *anterior lateral plate* (G. 2), springs from the outer side of the cochleariform process and adjacent part of the bar, and supports the anterior part of the inner wall of the labellum. To the concave margin formed by the edges of the lateral plates and the central portion of the bar the inner wall of the labellum is attached. In the resting position of the disc the lateral plates are directed downwards, but in the "scraping" and "direct feeding" positions they are rotated outwards on the bars. Their recoil towards the resting position probably plays some part in closing the labella.

A thin fold of chitin, arising from the inner side of the paraphysis and previously described as the inferior disco-haustellar fold, is continued along the inner side of the cochleariform process and terminates in the brush.

From the free surfaces (proximal, ventral and lateral) of the nodulus springs a structure which Lowne (p. 144) terms the *elastic ligament* (L.). A thick sheet of chitin, consisting of a clearly defined fibrillar core surrounded by a hyaline sheath, concave both transversely and longitudinally, stretches backwards from the nodulus as far as the extremities of the posterior lateral plates with whose inner margins it is fused. This sheet forms under the interlabellar membrane a strong floor for the prestomum in this region. By its elasticity this portion of the elastic ligament may aid in bringing the internal surfaces of the labella into apposition when feeding ceases. From the haustellar side of the nodulus a long, ventrally curved, rounded process of the same structure, fused centrally with the transverse sheet, depends and ends freely[1]. Portions of the labial salivary glands lie on either side of this process and their sheaths seem to enclose it, thus connecting the process to the skeleton of the labial gutter. When the oral disc is flexed on the haustellum in the resting position the process of the elastic ligament, which then lies close to the hypoglossa, shows a very distinct curve, but when the disc is extended the process is carried away from the hypoglossa. In this position the process is nearly straight, apparently owing to traction exerted, especially on its apical portion, by the encircling sheath of the gland. Hence this process seems to act as a spring tending to flex the disc, but it is not the chief agent in flexing the disc (Plate XIX, Text-fig. 14).

Viewed from the labellar surface (Text-fig. 14, II) the bars are seen to diverge from the nodulus for a considerable distance and then to bend inwards sud-

[1] Lowne thinks the elastic ligament is "connected with the furca," but no connection was found either in dissections or sections.



A portion of the right labellum and discal sclerite to show the relationships of the prestomal teeth. Inner row of prestomal teeth green, second row red, third row blue, fourth row yellow.

A. Flexural area of pseudotracheal membrane. *B*. Common anterior collecting channel. *C*. Brush. *D*. Cochleariform process. *E*. Anterior lateral plate of discal sclerite. *F*. Bar of discal sclerite. *G*. Dental area of pseudotracheal membrane. *H*. Posterior lateral plate of discal sclerite. *I*. Nodulus. *J*. Elastic membrane with fibrillar core (shaded). *K*. Common posterior collecting channel.

denly just in front of the paraphysal notch, leaving between them a triangular space, whose apex is the nodulus and base the overlapping inferior disco-haustellar folds, projecting inwards from the cochleariform processes, which lie parallel to each other. This space is the passage through which food passes into the labial gutter and is called by Hewitt (1914, p. 17) the *oral aperture* (see also Text-fig. 36).

(1) *The disco-labial joint.*

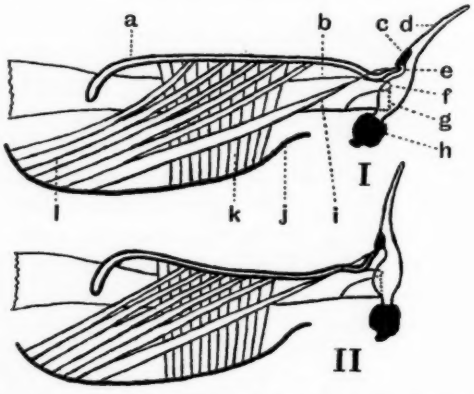
The distal end of the labial gutter is oval in section and slightly smaller than the oral aperture. Its ventral wall and most of its lateral walls are formed by the hypoglossa, which at its distal extremity is thin ventrally but strong laterally. The strong lateral portions or lateral cornua lie within the broad portions of the bars of the discal sclerite and the thin anterior portion within the apex of the oral aperture. A delicate, lax membrane unites the thin portion of the hypoglossa to the margin of the posterior region of the discal sclerite, allowing of the formation in some attitudes of a deep pouch, which Lowne (p. 144) terms the *poculum* (L.). The strong lateral cornua of the hypoglossa are attached to the inner surfaces of the discal bars by stronger, less lax membrane, and this syndesmosis forms the axis on which antero-posterior movement, the only one possible in this joint, occurs. The transverse grooves in the heads of the paraphyses fit, when the disc is fully extended, into the paraphysal notches in the discal bars in order to prevent over-extension, when the fly presses the disc against some surface, but this articulation does not form an axis about which movement takes place. The membrane uniting the hypoglossa to the discal sclerite is continued forwards uniting the paraphysis also with the discal sclerite, but its anterior edge is thickened to form a strong *lateral ligament* (G. 2) passing between the inner tubercle of the head of the paraphysis and the cochleariform process on each side. The arrangement of this membrane can be seen best in specimens in which the sclerites are drawn apart after removal of the surrounding structures. The inferior disco-haustellar folds, passing from the paraphyses to the cochleariform processes, also unite these structures.

Extension of the oral disc seems to be due to traction on the bases of the cochleariform processes through the lateral ligaments when the heads of the paraphyses are retracted by muscular action, and flexion to the elastic recoil of the paraphyses aided by the action of the cornua of the hypoglossa.

The retractors of the paraphyses by their direct fibres shorten the paraphyses by bending their middle thirds backwards and outwards and cause them to slide proximally on the lateral cornua of the hypoglossa, and by their anterior tendinous slips retract the heads. The extremities of the paraphyses are thus drawn upwards in relation to the lateral cornua of the hypoglossa, and through their ligaments traction is made on the cochleariform processes resulting in extension of the oral disc. The slightly thickened anterior edges of the lateral cornua seem during these movements to slide in grooves, which are shallow continuations of the transverse notches of the heads, in the

apposed sides of the paraphyses. The anterior fibres of the transverse muscle perhaps aid the retractors in bending the paraphyses.

When the muscles relax the paraphyses resume their normal positions and their heads, engaging in the paraphysal notches, push the cochleariform processes forward and so flex the disc. In fresh specimens from which all the muscles have been removed extension of the oral disc may be brought about



Text-fig. 15. Diagram to illustrate the mechanism for flexing and extending the oral disc. I. *Resting position*, in which the discal sclerite carrying the oral disc is flexed on the haustellum. (To avoid complication incomplete flexion is illustrated.) Flexion is brought about by recoil following muscular relaxation. During muscular contraction the paraphyses are bent, but when the muscles relax they straighten and their heads, pressing against the paraphysal notches, cause the discal sclerite to rotate on the distal cornua of the hypoglossa and assume a flexed position. II. *Extended position*. Extension is brought about by the traction on the bases of the cochleariform processes of the lateral ligaments, causing the discal sclerite to rotate on the distal cornua of the hypoglossa until it is at right angles to the haustellum. This traction is the result of muscular action. Contraction of the retractors of the paraphyses and of the transverse muscles bend, and therefore shorten, the paraphyses. Consequently their distal ends, to which the lateral ligament are attached, are caused to slide proximally on the distal cornua of the hypoglossa. Further, the anterior slips of the retractor muscles draw the heads of the paraphysis upwards and increase the traction on the ligaments.

a. Paraphysis. *b*. Hypoglossa. *c*. Lateral ligament. *d*. Cochleariform process. *e*. Paraphysal notch in bar of discal sclerite. *f*. Distal cornu of hypoglossa on which the discal sclerite rotates. *g*. Thin area between the cornua of the hypoglossa. In the flexed position this is embraced by the bars of the discal sclerite. *h*. Nodulus. *i*. Anterior slip of the retractor of the paraphysis. *j*. Longitudinal section of mentum. *k*. Transverse muscle of haustellum. *l*. Retractor of the paraphysis near its origin from the mentum.

either by pressing the paraphyses inwards towards the hypoglossa or by traction on the tendons passing to their heads, thus demonstrating the action of the muscles and the recoil of the paraphyses.

In the region of the hypoglossal articulations the inner walls of the discal bars, against which the hypoglossal cornua press from within when the disc is extended, are bevelled outwards towards the haustellar surface. In passing from extension to flexion of the disc the tips of the lateral cornua seem to

move from the narrower to the wider parts of the bevel, releasing tension and aiding flexion.

(2) *The superior and inferior mento-furcal joints.*

The two joints on each side, superior and inferior, which allow of movements of the furca on the mentum, lie in the ventral wall of the proboscis.

The *superior* is the joint between the mento-furcal bar and the fork of the mentum. Viewed from the surface each fork ends in an oblique margin, apparently parallel to the surface. The inner extremity of this margin is, however, much deeper than the outer for on dissection it is found that while both the strongly pigmented sides of the fork, called by Lowne the *inner and outer supports of the cornua*, are inclined from the plane of the mentum towards the hypoglossa, the inner dips much more steeply than the outer and inclines outwardly, so that the margin joining their extremities is nearly perpendicular to the surface of the haustellum. To this margin the proximal end of the mento-furcal bar is attached by thin chitin, forming a joint capable of great oblique-lateral movement, but not of direct antero-posterior movement. No muscles are attached to the mento-furcal bars and passive movements only occur at the superior joint in response to movements of the lateral processes of the furca, with which the distal ends of the bars articulate.

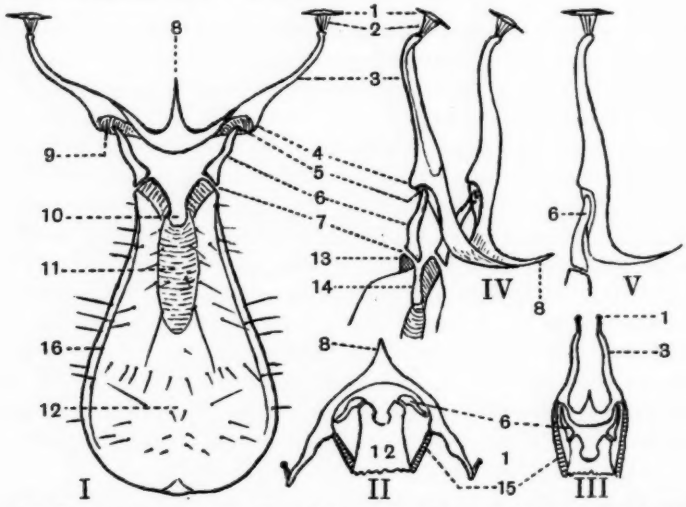
Viewed from the ventral surface the *mento-furcal* bars appear as thin rods, slightly bowed outwardly, ending proximally in broadened bases, which articulate with the forks, and distally in curved points lying in fossae in the lateral processes of the furca. Viewed laterally the bars appear as elongated, broad-based triangles. The integument of the depression between the forks (p. 52) is continuous with that between the inner margins of the bars, and distally is reflected on to the central process of the furca. The outer margins of the bars are continuous with the membranous lateral walls of the haustellum.

The *inferior mento-furcal joint* (G. 2) of each side lies between the mento-furcal bar and the lateral process of the furca.

The *furca* (L.) is shaped like a pair of "spring forceps," the arms, or *lateral processes* (L.), being strong rods in the outer walls of the labella, continued more than half way to the prestomal margins. The distal end of the lateral process is curved slightly outwards and is connected by a strap-like band of slightly pigmented chitin to the *epifurca* (L.), an elongated plate of strong, deeply-pigmented chitin, bearing macrochaetae, and lying in the long axis of the outer wall of the labellum. The posterior border of the lateral process is slightly curved, but the anterior bears on its proximal third a large *tubercle* (G. 2) for the insertion of the retractor muscle of the furca. Proximally to the tubercle there is a deep elongated *fossa* (L.), in which the distal end of the mento-furcal bar lodges, being attached by a ligament to the base of the distal part of the fossa[1]. The part of the furca corresponding to the *spring* (G. 2) of the

[1] Lowne (p. 143) thinks that in certain movements "the thyroid cornua are bent back and slide outwards in the grooves of the furca."

forceps is a thin, curved sheet of chitin, connecting the lateral processes and having a single, triangular, curved *central process* (L.), projecting backwards from it in the posterior wall of the disc.



Text-fig. 16. The mentum and furca. The relation of the mentum to the furca in (I) the "filtering position," in (II) the "scraping position" and in (III) the "resting position," viewed from the posterior surface. IV. Oblique view of the furca, and distal part of the mentum. V. Side view of the furca in the resting position.

1. Epifurca. *2.* Strap-like band of chitin joining the epifurca to the lateral process of the furca. *3.* Lateral process of furca. *4.* Tubercle on the lateral process for the insertion of the retractor of the furca. *5.* Inferior mento-furcal joint. *6.* Mento-furcal bar. *7.* Superior mento-furcal joint. *8.* Median process of furca. *9.* Fossa in the lateral process of the furca to accommodate the distal end of the mento-furcal bar. *10.* Semicircular sinus of mentum with the forks on either side. *11.* Groove in the mentum. *12.* Body of mentum. *13.* Inner support of cornu. *14.* Outer support of cornu. *15.* Retractor muscle of furca. *16.* Lateral ridge of mentum.

In the resting position the lateral processes of the furca are parallel with each other, but by the action of the retractor muscles they may be bent back to lie in one plane or even at an obtuse angle (Text-fig. 35). In these movements the mento-furcal bars are flexed outwards on the forks at the superior mento-furcal joints, finally pointing almost backwards instead of forwards, and their distal extremities act as fulcra on which the lateral processes of the furca rotate, until the original concavity of the central spring-like portion has been rendered convex.

The return of the lateral processes to the resting position, carrying with them the labella, is probably due to the recoil of the central spring. If the furca is dissected out from a recently killed specimen the lateral processes return to the parallel position after separation, showing that the forks of the mentum are not, as Lowne (p. 393) suggested, responsible for the recoil.

The mento-furcal joints are constructed to perform the lateral movements necessary for separating the labella, but passive antero-posterior movement occurs at the inferior joint, when the disc is flexed or extended on the haustellum.

(3) *The inner wall of the labellum or pseudotracheal membrane.*

The inner wall of the labellum consists of a thin, almost transparent, chitinous membrane attached centrally to the bar of the discal sclerite and laterally along the edges of its lateral plates, and continuous at the prestomal margin with the outer wall. Its proximal or *dental area* (G. 2), which lies within the semicircle formed by the lateral plates of the discal sclerite, bears the prestomal teeth, beyond this a more flexible portion, the *flexural area* (G. 2), supports the collecting channels of the central set of pseudotracheae, and distally it is modified into thin plates and grooved by the pseudotracheal channels. Posterior to the discal sclerite the inner walls of the two labella unite to form the *interlabellar membrane.*

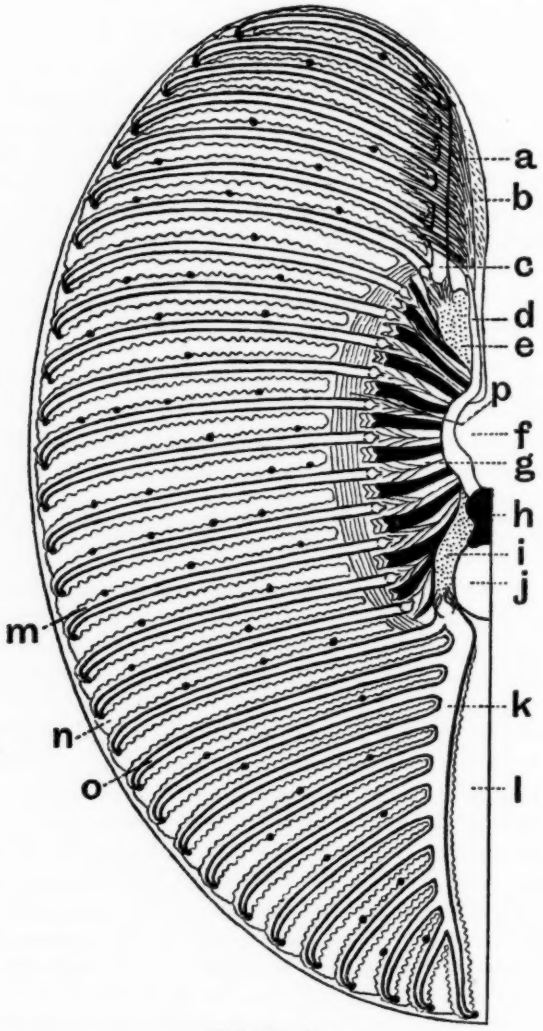
(4) *The pseudotracheae* (Suffolk, 1869).

Each pseudotrachea, together with its lateral appendages, the interbifid grooves, is supported by a thin chitinous *pseudotracheal plate* (G. 2), slightly curved in the same direction as the pseudotrachea at its outer extremity, and attached laterally to *interpseudotracheal plates* (G. 2), by thin chitinous syndesmoses. Viewed from the surface these syndesmoses appear as pairs of parallel wavy lines between the pseudotracheae. The distal end of each pseudotrachea lies near the margin of its pseudotracheal plate. Between the prestomal margin and the terminations of the pseudotracheal and interpseudotracheal plates small angular plates are developed in the pseudotracheal membrane. Proximally the pseudotracheal plates fuse with the thin chitin of the flexural area. (Text-fig. 18.)

The pseudotracheae, varying in number between 28 and 34, run transversely across the labellum. From its origin near the prestomal margin, where its terminal ring surrounds a "gustatory papilla" (Text-figs. 17, 18), each runs in a small semicircle before commencing its transverse course.

The pseudotracheae may be divided into three sets. The anterior set run into a common *anterior collecting channel* (G. 1), which opens anterior to the prestomal teeth. Similar, the posterior set run into a common *posterior collecting channel* (G. 1), which opens posterior to the prestomal teeth. The pseudotracheae of the central set terminate in the flexural area in short tubular portions or *central collecting channels* (G. 2), which open between the prestomal teeth, without the intervention of common collecting channels. Grooves, or *gutters* (G. 1), whose lateral walls are formed by the prestomal teeth, continue the lines of the central collecting channels in the dental area down to the margin of the discal sclerite. By this arrangement all the pseudotracheae are made to converge to the discal sclerite, which bounds the oral aperture.

The pseudotracheae are deep, tubular, incomplete furrows embedded in the substance of the inner wall of the labellum, which project sufficiently to produce distinct ridges. Along the apex of the ridge the wall of the

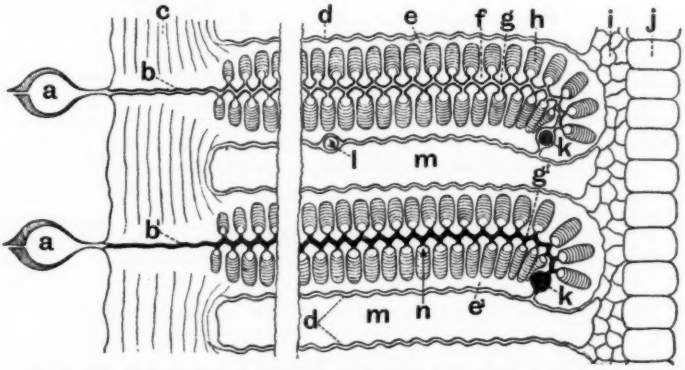


Text-fig. 17.

pseudotrachea is lacking so that the interior of the tube is in communication with the oral surface of the disc through a very narrow *zigzag fissure* (G. 1). The lumen of the tube is kept open by means of incomplete, deeply pigmented, chitinous rings running transversely round the tube, each of

Text-fig. 17. Left labellum spread out to show the six anterior pseudotracheae running into the common anterior collecting channel (*c*), the twelve central pseudotracheae opening independently by their individual collecting channels between the prestomal teeth, and the twelve posterior opening into the common posterior collecting channel. The prestomal teeth of the inner row (black) act as sides to the "gutters" conveying the fluid food from the openings of the central pseudotracheae to the oral orifice, while the "fangs" of the outer rows act as floors. The positions of the gustatory papillae are indicated by black dots. There is one in association with the distal end of each pseudotrachea, and the others open through the syndesmoses between the pseudotracheal (*m*) and interpseudotracheal (*n*) plates. The numbers in the interspaces between adjacent pseudotracheae vary from none to four.

a. Brush covering the common anterior collecting channel. *b*. Hair tract. *c*. Common anterior collecting channel. *d*. Cochleariform process. *e*. Anterior lateral plate of discal sclerite. *f*. Oral aperture. *g*. "Gutter" of one of the central pseudotracheae. The "fangs" of the teeth are embedded in the "dental area" of the pseudotracheal membrane. *h*. Nodulus. *i*. Posterior lateral plate of discal sclerite. *j*. Elastic ligament. *k*. Common posterior collecting channel. *l*. Inner surface of interlabellar membrane. *m*. A pseudotracheal plate. *n*. An interpseudotracheal plate. *o*. One of the pseudotracheae. *p*. Flexural area of pseudotracheal membrane.



Text-fig. 18. A portion of the inner (pseudotracheal) surface of the labellum, supporting two central pseudotracheae, with their distal ends to the right. The upper is represented with the longitudinal zigzag fissure open, the lower with it closed, as it is in the act of feeding.

a. Opening of the collecting channel between the prestomal teeth. *b*. Longitudinal fissure of the collecting channel open; b^1, closed. *c*. Thin membrane of the flexural area, replacing the pseudotracheal and interpseudotracheal plates in the region of the collecting channel. *d*. Syndesmosis between the pseudotracheal and interpseudotracheal plates. *e*, e^1. Pseudotracheal plates. At their proximal ends they merge into the thin membrane, *c*. *f*. A prominence between two interbifid grooves, produced by a fold of the integument enclosing the flattened end of a pseudotracheal ring and the extremities of the adjacent forks on each side of it (see Text-fig. 21). *g*. Longitudinal zigzag fissure of a pseudotrachea open; g^1, closed. *h*. One of the interbifid grooves. *i*. Small angular plates lying between the pseudotracheal and interpseudotracheal plates and the row of larger quadrilateral plates (*j*) at the prestomal margin. *j*. Single row of slightly pigmented, quadrilateral plates in the *outer* wall of the labellum. *k*. Terminal gustatory papilla enclosed by the distal end of a pseudotrachea. *l*. A gustatory papilla projecting through the syndesmosis. *m*. Interpseudotracheal plate. *n*. Arrow showing the direction in which fluid is sucked along an interbifid groove and through an interbifid space into a pseudotrachea.

The prestomal margin is at the junction of *i* and *j*.

which has one fork-like bifid extremity, enclosing a rounded space between the prongs, and one extremity slightly expanded and flattened so as to resemble the tail of a fish. The rings are arranged in such a manner that along each side of the central fissure the bifid extremity of one ring alternates with the expanded extremity of the next ring. In consequence of this arrangement, which is very clearly seen in the labella of freshly emerged flies or in preparations treated with potash, the margin of the pseudotrachea at each side of the central fissure has a deeply indented or scalloped appearance. The really

Text-fig. 19.

Text-fig. 20.

Text-fig. 19 is a side view of a pesudotracheal ring. At its right-hand end the ring has a flattened expanded extremity; at its left-hand end a bifid extremity. The opening of the longitudinal fissure is seen between the flattened end of the ring and the tips of the forks. The arrangement of the fold of integument forming the interbifid groove is indicated by means of shading. (From Graham-Smith, *Journ. of Hyg.* 1911.)

Text-fig. 20 represents two consecutive pseudotracheal rings, showing the relationship of their bifid and flattened extremities, as seen from the oral surface of the disc.

effective entrances into the pseudotracheae are through the spaces between the bifid extremities of the rings and not through the narrow continuous zigzag fissure, which is at any time extremely narrow and is closed during the act of feeding, as will be explained later.

The pseudotracheae diminish slightly in diameter as they approach the margin of the disc, and the sizes of the forked extremities of the rings and consequently of the spaces between them also diminish though not to a corresponding degree.

The term *interbifid space* (G. 1) is used to indicate the area enclosed between the forks of the bifid extremity of a ring.

Text-fig. 20 illustrates two consecutive rings with their bifid and flattened extremities, and Text-fig. 19 illustrates a side view of one of these rings. Pl. XXI, figs. 13, 14, are photographs of two rings and fig. 16 of several consecutive pseudotracheal rings which have been treated with potash and compressed. The terminations of the consecutive rings are well shown. Pl. XXI, fig. 15, is a photograph of the oral surface of the disc of a blow-fly after treatment with potash showing portions of four pseudotracheae. The longitudinal fissures of the pseudotracheae and the forked extremities of the rings and

interfibid spaces can be clearly seen. Pl. XXI, fig. 17, is a photograph of a section of the disc showing four pseudotracheae cut transversely. The chitinous rings, the openings of the longitudinal fissures and the ridges caused by the projection of the tubes above the surface are clearly shown.

At their proximal extremities the pseudotracheae discharging into the common anterior and posterior collecting channels unite with them nearly at right angles without change of structure, but each central pseudotrachea becomes transformed into a central collecting channel. The extremities of the rings cease to be bifid, but are slightly expanded and the pseudotrachea is modified into a tube without interbifid spaces (Text-fig. 23), which conducts the contained fluid to the opening between the extremities of two sets of super-imposed prestomal teeth.

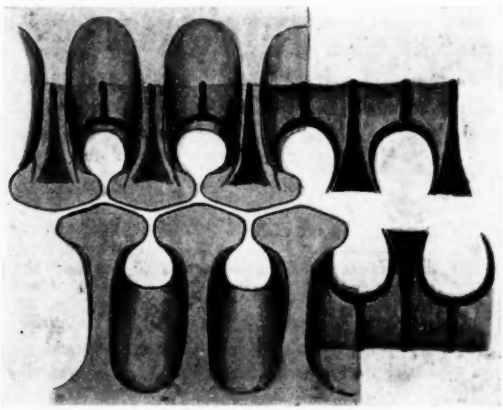
The pseudotracheae of several of the common non-biting flies closely resemble each other, though they exhibit slight and presumably unimportant differences in their structure. The average measurements of the various parts in six common species are as follows:

	Pseudotracheae		Interbifid spaces	
	Diameter at proximal end (mm.)	Diameter at distal end (mm.)	Diameter near the proximal ends of the pseudotracheae (mm.)	Diameter near the distal ends of the pseudotracheae (mm.)
Calliphora erythrocephala	·02	·01	·006	·004
Sarcophaga carnaria ...	·02	·01	·005	·004
Lucilia caesar	·02	·01	·006	·004
Fannia (*Homalomyia*) *canicularis*	·016	·008	·006	·004
Ophyra anthrax ...	·016	·008	·006	·004
Musca domestica ...	·016	·008	·004	·003

The cuticle lining the oral surface of the labellum dips down into the pseudotracheae through the longitudinal fissure and also forms the lining of these tubes, as may be seen by reference to Pl. XXI, fig. 17. In passing downwards into a pseudotrachea the cuticle accurately follows the supporting rings, being closely adherent not only to the chitinous sides of the interbifid spaces but also to the intervening elevations between them produced by the projection of the expanded ends of the alternate rings, and the application to them of the adjacent forks of the neighbouring rings on either side.

Owing to this arrangement a remarkable series of folds is produced in the cuticle forming channels or grooves leading into the interbifid spaces. If the cuticle is traced along the edge of the longitudinal fissure from the bottom of one interbifid space to the bottom of the next it can be observed to be very closely attached to the chitin along the base of an interbifid space and up the side of a fork to its pointed extremity. It then passes over the expanded portion of the alternate ring and down the adjacent fork of the next ring, binding the two forks mentioned and the expanded portion of the intermediate ring into an elevated mass which lies between the deep depressions of the interbifid

spaces. The arrangement described can be most easily understood by reference to Pl. XXI, fig. 18, a longitudinal section through a pseudotrachea just to one side of the central fissure, and Text-fig. 22. The depressions caused by the cuticle adhering to the bases of the interbifid spaces are continued outwards as folds or grooves in the cuticle almost to the margins of the pseudotracheal plates. Each *interbifid groove* (G. 1) thus forms a well-defined channel leading into the pseudotrachea through the interbifid space with its long axis at right angles to the line of the pseudotrachea. The deepest part of the groove is at its entrance into the pseudotrachea, and at this point it loses its groove-like



Text-fig. 21 represents a dissection of a portion of a pseudotrachea. On the right the integument of the oral surface of the labellum has been removed so as to show a portion of the pseudotrachea with the alternate bifid and flattened extremities of the rings and the membrane lining the interior of the tube stretching between them. On the left lower portion the appearance of the surface integument is represented. Two interbifid grooves leading to their interbifid spaces are shown. Between the spaces are elevated masses, each of which is produced by a fold of the integument enclosing the flattened end of a ring and the extremities of the adjacent forks on each side. In the left upper portion of the diagram is shown the appearance of those structures as seen by transmitted light so as to indicate more closely the relationship of the integument to the rings.

character and becomes a tunnel, though still communicating with the surface by a very narrow slit. When the proboscis is erected by slight pressure on the head and the oral sucker viewed with a microscope these grooves can be easily seen as regularly placed channels running at right angles to each pseudotrachea. (Text-fig. 18.)

Though difficult to describe the arrangement can be easily understood by reference to Text-fig. 21, representing a dissection of a portion of a pseudotrachea. On the right-hand side the integument of the oral surface of the labellum has been removed so as to show a portion of the pseudotrachea with the alternate bifid and flattened extremities of the chitinous rings and the membrane lining the interior of the tube stretching between them. On the

left-hand lower portion the appearance of the surface integument is represented. Two interbifid grooves leading to their interbifid spaces are shown. Between the interbifid spaces are elevated masses, each of which is produced by a fold of the integument enclosing the flattened end of a ring and the extremities of the adjacent forks on each side. In the left-hand upper portion of the diagram is shown the appearance of these structures as seen by transmitted light so as to indicate the relationship of the integument to the rings.

In Pl. XXI, fig. 17, illustrating transverse sections of four pseudotracheae, the interbifid groove is indicated on the left side of each pseudotrachea, but is perhaps best seen in the central ones. In each case the point of bifurcation of the chitinous ring is indicated by a dark spot above which the forks are curved inwards. From the point of bifurcation a distinct line, which represents the reflection of the cuticle at the base of the interbifid groove, passes obliquely upwards and outwards to the surface of the integument. Text-figs. 19 and 20, IV, illustrate diagrammatically the condition seen in transverse sections.

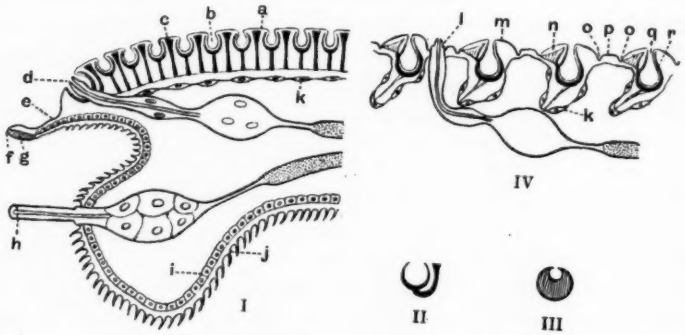
Anthony (1874), Wright (1884) and Lowne (1892, p. 395) all regarded the interbifid grooves as suckers. The latter figured them as blind sacs attached to the forks of the rings with openings into the pseudotracheae only. None of these authors seemed to regard them as channels leading into the pseudotracheae.

The fact that these interbifid grooves are really channels leading into the pseudotracheae can be demonstrated, however, by a very simple experiment. If the labellum of a blow-fly is hardened in alcohol, formalin or other preserving agent, and is later mounted in water under a cover-glass and examined with the aid of a microscope the grooves can be clearly seen. As the specimen begins to dry air bubbles often form in the slight depressions on the oral surface of the disc between the pseudotracheae. As the drying continues the bubbles run into the interbifid grooves and through them into the pseudotracheae, clearly showing that the grooves lead into the pseudotracheae.

(5) *The gustatory papillae.*

Projecting through the syndesmoses between the pseudotracheal and interpseudotracheal plates may be seen the terminations of sense organs. Lowne (1870, p. 49) at first regarded them as "organs of taste," but later (1892, p. 393) thought that they were "the orifices of the ducts of the labial glands." Hewitt (1914) calls them *gustatory papillae.* It seems likely from their position and resemblance to tactile setae that these organs may have a tactile as well as a gustatory function. The projecting chitinous end of the papilla is nipple-shaped and pierced by a fine duct, which ends in a pyriform mass of sensory cells, in which a branch of the labial nerve terminates. Between adjacent pseudotracheae there may be none, one, two, three or four such papillae (see Text-fig. 17). According to Lowne (1892, p. 393) "there are usually five or six in each interspace," and Hewitt (p. 17) states that in *M. domestica* there are "generally four or five in each interpseudotracheal area." Beside these

there is one, which may be termed the *terminal gustatory papilla* (G. 2), situated at the distal end of each pseudotrachea. The distal portion of this organ passes through the syndesmosis, where the latter dips into the pseudotracheal plate (Text-fig. 18) and projects into the lumen of the pseudotrachea, apparently entering through the enlarged bifid extremity of the last pseudotracheal ring (Text-fig. 22). Its tip reaches the surface through a small dilatation in the longitudinal fissure of the pseudotrachea.



Text-fig. 22. I. Transverse section through the edge of the labellum in the "filtering position." II. Modified terminal pseudotracheal ring which embraces a "terminal gustatory papilla." III. Section through a "gustatory seta." IV. Longitudinal section through the pseudotracheal surface of the labellum, showing four pseudotracheae in transverse section.

a. Integument binding together the flattened extremity of a pseudotracheal ring and the prongs of two adjacent forks, and dipping down into the interbifid space. *b*. Interbifid space in section. *c*. Flattened extremity of a pseudotracheal ring. *d*. Terminal gustatory papilla passing through the modified last interbifid space. *e*. Area of angular plates. *f*. Prestomal margin. *g*. Quadrilateral plate at the prestomal margin. *h*. Gustatory seta. *i*. Hypodermis. *j*. Chitinous cuticle. *k*. Sub-pseudotracheal membrane, with thin fibrils passing to it from the deep aspect of each pseudotrachea. *l*. Gustatory papilla projecting through the syndesmosis between a pseudotracheal and an interpseudotracheal plate. *m*. A part of a pseudotracheal plate. *n*. Interbifid groove (in longitudinal section). *o*. Syndesmosis between a pseudotracheal and an interpseudotracheal plate. *p*. Interpseudotracheal plate. *q*. Opening of zigzag fissure. *r*. Integument lining the pseudotrachea, and continuous with the surface integument.

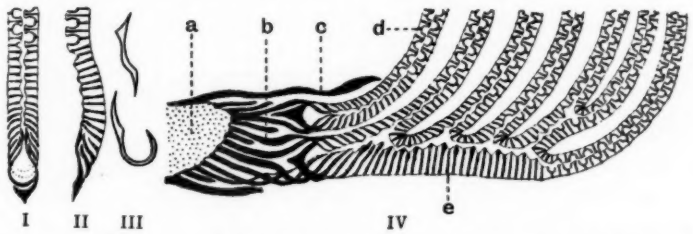
(6) *The collecting channels.*

The collecting channels, which are kept open by incomplete chitinous rings, whose extremities are either quite plain, or slightly expanded, or possess only the rudiments of forks, communicate with the surface of the labellum by narrow fissures, which are continuations of the longitudinal zigzag fissures of the pseudotracheae. During feeding and probably at other times the edges of these fissures are in apposition and no fluid can enter through them. Since there are no interbifid spaces there are no interbifid grooves, and the collecting channels function as conducting tubes.

The *collecting channels of the central set of pseudotracheae* are short and appear to be straight, when viewed from the surface of the labellum. In

transverse sections, however, a decided outward bend, the *dental flexure* (G. 2) is visible. The dental flexure, which is due to the disposition of the chitinous rings has the important function of enabling the inner surfaces of the two labella to meet beyond the prestomal teeth, both in the resting position and when the oral disc is spread out in the "filtering" and "cupping" positions (Text-fig. 35). It is only when the tendinous cords exert traction on these collecting channels that the bends are obliterated and the teeth exposed in the "scraping" and "direct-feeding" positions. The recoil of these channels may play some part in the return of the labella to the resting position.

The distal and central rings of these channels, which have blunt, non-bifid extremities, are, like those of the pseudotracheae, perpendicular to the surface of the pseudotracheal membrane, but the proximal rings are disposed at decreasing angles until the terminal rings come to lie parallel with it, and the



Text-fig. 23. The chitinous rings of the collecting channels. I. Oral view of a collecting channel of a central pseudotracheae. II. Side view of same to show the dental flexure. III. Two terminal rings, resembling lateral teeth of the third row. IV. Common anterior collecting channel.

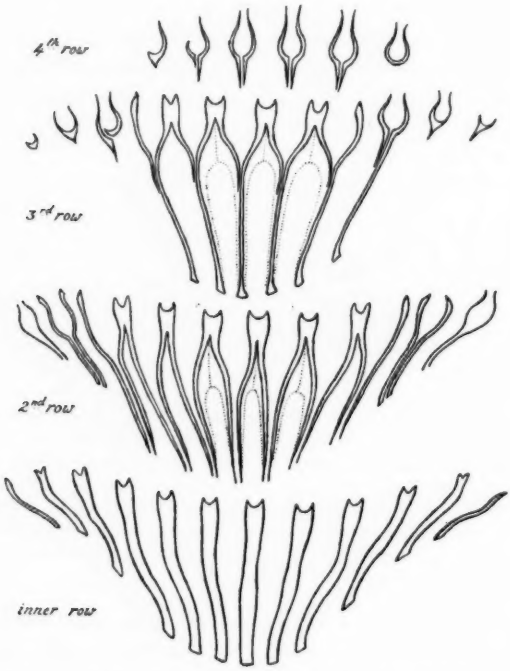
a. Portion of the anterior lateral plate of the discal sclerite breaking up into chitinous rods (*b*), which are transformed distally into pseudotracheal rings. *c*. Anterior prestomal tooth of inner row. *d*. Anterior pseudotrachea of the central set. *e*. Common anterior collecting channel, with six pseudotracheae opening into it.

passages into the mouths of the channels are in consequence at right angles to the surface. The mouths of these collecting channels lie between the tips of the prestomal teeth, and the terminal rings are much modified, being often irregular, flattened or asymmetrical. In fact, the conversion of rings into prestomal teeth may be traced (Pl. XIX). The portion of the pseudotracheal membrane which supports the central collecting channels has been termed the *flexural area*. Beyond the mouth of the channel a *gutter*, bordered by superimposed teeth and floored by the dental area of the pseudotracheal membrane, in which the "fangs" of the outer rows of teeth are embedded, continues its line towards the oral aperture.

The *common anterior and posterior collecting channels* are constructed on the same plan as the central collecting channels, but at their proximal ends the rings are gradually modified into broad longitudinal bars, so disposed as to form shallow gutters, continuing the lines of the collecting channels. The proximal ends of the bars are fused with the lateral plates of the discal sclerite.

(7) *The prestomal teeth.*

The *prestomal teeth* (L.) are chitinous prong-like processes, free at their distal, bifid extremities, but with their proximal portions embedded in the dental area of the pseudotracheal membrane. Their proximal ends articulate, depending on their position, either with the bars or with the lateral plates of the discal sclerite in such a manner that the teeth can be abducted outwards,



Text-fig. 24. The four rows of prestomal teeth separated to show their "fangs," which are thickenings in the dental area of the pseudotracheal membrane. The dotted lines below the central teeth of the second and third rows indicate delicate plates, or *flanges*, below the junctions of the fangs, to which the looser portions of the dental membrane are attached.

but antero-posterior movement is limited. In the centre of the labellum there are from within outwards four rows, so arranged that their free extremities are superimposed, but laterally the outer two rows are lacking, though represented by modified rings at the mouths of the collecting channels.

The *first* or *inner row* is the strongest and most complete, consisting of from 10 to 12 well-formed teeth. Each tooth is a thin, slightly curved slip of chitin with a flattened side presenting on the inner surface. In the broad, but extremely thin, distal end there is a deep notch so that the tooth terminates in

a crescent with sharp points[1]. Viewed from the outer, or ab-oral, surface the lateral borders of the proximal half of each tooth seem to be thickened, leaving between them a V-shaped depression to the margins of which the dental portion of the pseudotracheal membrane is attached. Apart from this attachment and its proximal articulation to the discal sclerite the tooth is free. The lateral thickenings represent the "fangs" of the other rows of teeth, and the thin chitin at the base of the V-shaped depression represents the delicate flanges projecting from their fangs.

The *second row* consists of from 6 to 8 fully developed teeth centrally, and partially developed teeth laterally. The distal thirds of the fully developed teeth resemble those of the inner row, but their "fangs," constituting their proximal two-thirds, separate and pass laterally behind the adjacent teeth of the first row to articulate with the sides of the fangs of the third row. Each fang has a delicate *flange* (G. 2) of chitin projecting laterally from it. The lateral teeth of the row are represented by fangs which fail to unite distally and terminate in single points, which are either needle-like in the most lateral or shaped like the bisected ends of fully developed teeth in the less lateral. Only the distal thirds of the teeth of this row are free, the pseudotracheal membrane being attached to the fangs.

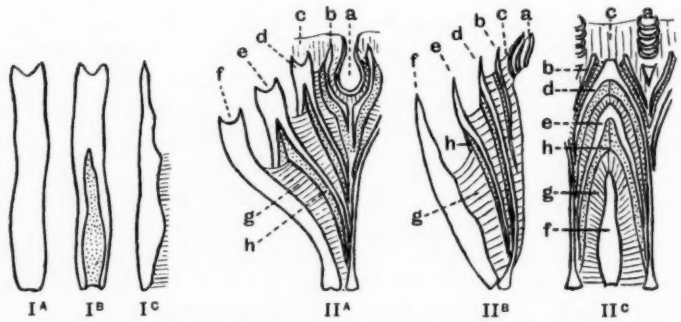
The *third row* consists of from 4 to 6 fully developed teeth centrally. These resemble the central teeth of the second row, except that one fang of each tooth is continued proximally to articulate with the discal sclerite and to afford near its base articular surfaces for the fangs of the second row, whilst the other fang is much shorter and articulates with the long fang of a neighbouring tooth of this row. The most lateral teeth of this row are slightly modified rings of collecting channels. In this row only the distal one-sixth of the tooth is free.

The *fourth and outermost row* is very slightly developed, being represented by 4, 5 or 6 modified terminal rings of collecting channels with very fine points. Only the points are free.

From their structure, position and relationship to the rings of the collecting channels the teeth seem to be modified pseudotracheal rings. The relation of the pseudotracheal membrane to the teeth may be demonstrated by pulling them forwards (Text-fig. 25) or by exposing their ab-oral surfaces by dissecting away the outer wall of the labellum and adjacent structures. From the edges of the V-shaped depressions in the ab-oral surfaces of the inner row of teeth the supporting *dental* portion of the pseudotracheal membrane passes as a loose fold to the delicate expansions or *flanges* from the inner lateral margins of the fangs of the second row. In like manner from the outer edges of the fangs of the second row the membrane passes to the flanges from the inner margins of the fangs of the third row, and similarly from the third to the fourth row. The fourth row and the adjacent proximal rings of the collecting

[1] Patton and Cragg (1913, p. 44) state that the free margins are "finely serrated," but in *C. erythrocephala* no serrations can be seen, even with an oil immersion lens.

channels are embedded in a continuation of the dental membrane which is continuous with the flexural membrane supporting the collecting channels. It will be seen that the fangs of the teeth in a superimposed series are united by loose membrane and concertina-like movement is possible.



Text-fig. 25. Prestomal teeth. I. A central prestomal tooth of the inner row. I A. Oral surface. I B. Ab-oral surface, showing the depression from the margins of which a fold of the dental membrane arises. I C. Side view, with an indication of the fold of the membrane. II. A superimposed series of four prestomal teeth. II A. View from the oral surface, with the teeth pulled inwards and slightly to the left. II B. Lateral view with the teeth pulled inwards. II C. View from the ab-oral surface. The free portions of the teeth which project on the oral surface are invisible.

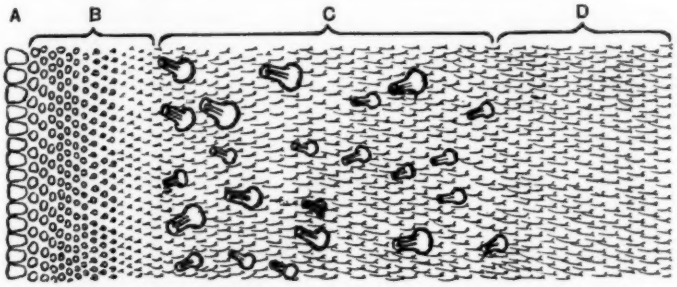
a. Mouth of the collecting channel of a central pseudotrachea. *b*. Tooth of fourth row. *c*. Part of dental membrane distal to the teeth. *d*. Tooth of third row. *e*. Tooth of second row. *f*. Tooth of inner row. (In II C only the ab-oral depression of tooth *f* and the fangs of teeth *b*, *d*, *e* are seen.) *g*. Loose fold of membrane passing from the edges of the ab-oral depression in tooth *f* to the delicate flanges (*h*) passing inwards from the fangs of tooth *e*. The membranes and expansions connected with the other teeth are indicated by similar shading.

When the fly desires to scrape a surface it can bring into action four rows of teeth on each side of the oral aperture, the first row having about 22, the second 22, the third 20 and the fourth 10 points, or a total of about 150 very fine blades capable of cutting in an antero-posterior direction. In fact the scraping apparatus of the fly resembles closely, both in structure and action, the machine known as a scarificator (Pl. XXII, fig. 8), formerly used for making incisions in the skin before cupping, in which several buried blades, operated by a spring, were protruded simultaneously through parallel slots in a flat surface. No more efficient apparatus than the fly's proboscis in the "scraping position" could be devised for intra-cutaneous, -conjunctival, or -mucous inoculation (Text-fig. 36, I).

(8) *The external wall of the labellum.*

The external wall of the labellum consists of a thin, chitinous membrane on which, except in the marginal strip, short, closely-set, curved hairs are present. Along the prestomal margin (Figs. 2. *14*; 26) is a single row of quadrilateral, slightly pigmented scales, whose edges form a very slightly

sinuous line (Text-fig. 18, *j*). Beyond the scales lie about ten rows of small, thin, rounded, slightly pigmented plates, gradually diminishing in size, the outer three developing minute curved hairs. This is the *marginal strip* (Text-fig. 2, *1*). Beyond this region the scales are small and form the broadened bases of the closely-set, curved, short hairs. In the area described as the *marginal fold* (Text-fig. 2, *2*) numerous larger and smaller grooved macrochaetae (Text-fig. 26), with large rings of thick chitin at their bases, arise amongst the smaller hairs.



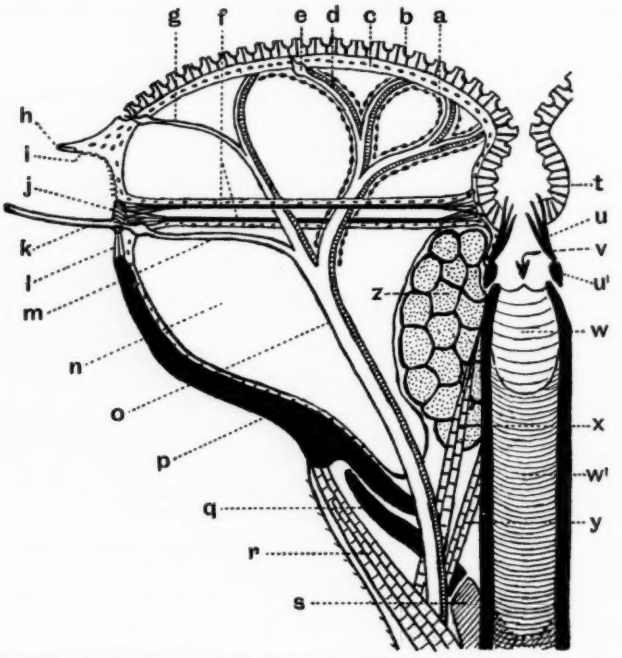
Text-fig. 26. A portion of the outer wall of the labellum, showing the prestomal margin on the left. *A*. Row of quadrilateral scales along the prestomal margin. *B*. Marginal strip. *C*. Marginal fold, with macrochaetae (gustatory setae) cut off shortly above their origins. *D*. A small portion of the wall beyond the marginal fold.

XI. THE INTERNAL ANATOMY OF THE LABELLUM.

The internal anatomy of the labellum is best demonstrated by the dissection of specimens fixed by various methods, while the oral discs are moderately distended by pressure on the head. If a specimen prepared in this way is divided by a longitudinal incision at right angles to the long axis of the oral disc, the more important structures within the labellum are visible, namely the hypoderm underlying the integument of the outer wall, the modified portion of it, subsequently called the sub-pseudotracheal membrane, under the inner wall, the tendinous cords, the labial salivary gland, the tracheae and the labial nerve (Text-fig. 27). In order to ascertain the structure and relationship of these organs dissections from various aspects of several specimens, fresh and preserved by various methods, are necessary, as well as the examination of serial sections cut in various planes.

(1) *The tendinous cords.*

Although Lowne (1870, p. 49) was the first to mention the tendinous cords stating that "the purpose of these cords seems to be to connect the extremity of the anterior processes of the mentum with the margins of the fissure between the lobes, which is probably kept closed by the elasticity of the pillars which support the orifices of the false tracheae, and which is opened by the muscles attached to the anterior processes of the mentum, through the agency of these

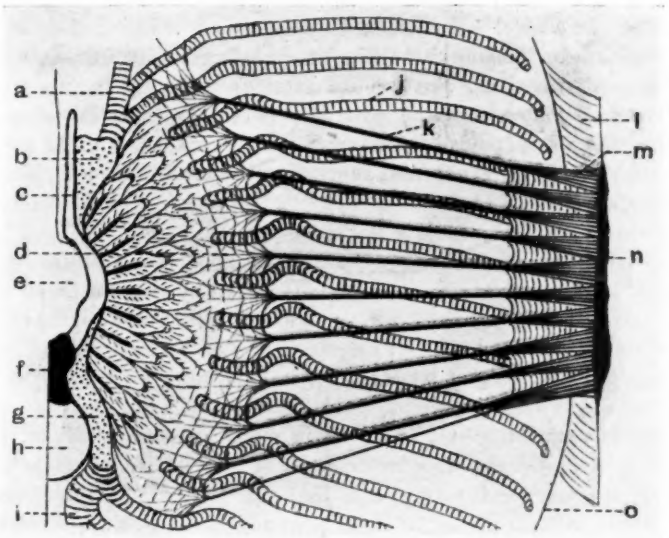


Text-fig. 27. Semi-diagrammatic longitudinal section through the left labellum at right angles to its long axis, while distended by slight pressure on the head.

a. Layer of pigmented cells (*pigmented membrane*) covering the labial nerve and its branches. Only those on the inner branch are represented. *b*. A central pseudotrachea (in longitudinal section). *c*. Sub-pseudotracheal membrane. Reflections are shown covering both surfaces of the tendinous cords. *d*. A trachea (shaded) accompanying a branch of the labial nerve. *e*. Gustatory papilla, in which a branch of the labial nerve terminates. *f*. Two of the tendinous cords, arising from the epifurca and inserted into the outer aspect of the flexural area of the pseudotracheal membrane. *g*. A branch of the labial nerve passing to a "terminal" gustatory papilla. *h*. Prestomal margin. *i*. Marginal strip. *j*. Epifurca in transverse sections. *k*. Macrochaeta arising from the epifurca. *l*. Strap-like band of chitin (Fig. 2, *20*), which connects the epifurca and the end of the lateral process of the furca. *m*. A branch of the labial nerve supplying a macrochaeta. *n*. Haemocoel. *o*. Trunk of labellar portion of labial nerve with its accompanying trachea (shaded). *p*. Lateral process of furca. *q*. Mento-furcal bar. *r*. Retractor muscle of furca. *s*. Fork of mentum. *t*. Dental flexure in the collecting channel of a central pseudotrachea. *u*. Prestomal teeth, articulating with the bar of the discal sclerite (*u'*). *v*. Arrow pointing to the "oral aperture." *w*. Semicircular area of thin chitin between the cornua of the hypoglossa. *w'*. Central plate of hypoglossa. *x*. Anterior slip and *y*. A proximal slip of the retractor of the paraphysis. *z*. Labial salivary gland.

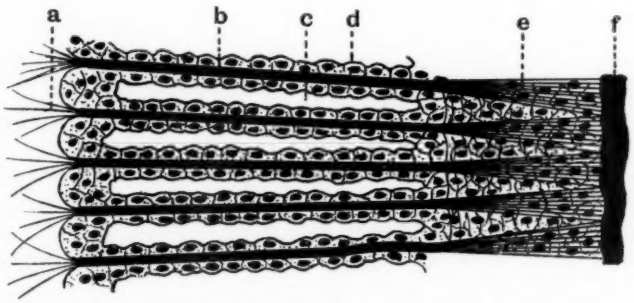
tendinous bands," and Kraepelin (1883) figures them, subsequent workers have paid little attention to them.

The *tendinous cords* (L.) of each labellum (Text-fig. 27) arise from the inner aspect of the epifurca, as fan-shaped collections of fibrils, united by a thin membrane. Shortly the fibrils unite to form 10 to 12 separate cords. These



Text-fig. 28. Internal anatomy of the oral disc. A dissection of part of the right labellum, after the removal of the greater part of its outer wall, to show the tendinous cords.

a. Common anterior collecting channel. *b*. Anterior lateral plate of discal sclerite. *c*. Cochleariform process. *d*. Dental area of the pseudotracheal membrane, showing the embedded "fangs" of the teeth. *e*. Oral aperture, *f*. Nodulus. *g*. Posterior lateral plate of discal sclerite. *h*. Elastic ligament. *i*. Common posterior collecting channel. *j*. A pseudotrachea, showing the dental flexure. *k*. One of the tendinous cords. *l*. Portion of the outer wall of the labellum turned aside. *m*. Fibrillar origin of the tendinous cords from the epifurca. *n*. Epifurca. *o*. Prestomal margin.



Text-fig. 29. Stained preparation of five tendinous cords, showing the reflection of the sub-pseudotracheal membrane covering them.

a. Fibrillar terminations of the cords fusing with the flexural area of the pseudotracheal membrane. *b*. One of the cords (black). *c*. Space between two cords. *d*. Cells of sub-pseudotracheal membrane surrounding a cord. *e*. Fibrillar origins of cords, covered by cells. *f*. A portion of the epifurca.

cords pass across the central blood-space and again unite behind the collecting channels of the central pseudotracheae to form an interlacing sheet attached mainly to the flexural region of the pseudotracheal membrane, but sending fibres to different destinations. Some fibres pass (*a*) anteriorly to fuse with the pseudotracheal plates, some (*b*) inwards between the collecting channels to fuse with the elevated folds between their mouths, and some (*c*) the strongest, fusing with the dental membrane, supporting the prestomal teeth, pass down to the discal sclerite.

The tendinous cords, which in the "intermediate," "scraping" and "direct-feeding" positions function as a fenestrated aponeurosis of the retractor muscle of the furca, transmit the traction exerted by this muscle on the epi-furca to all parts of the pseudotracheal membrane lying between the collecting channels and the discal sclerite, but have no direct influence on the distal portion supporting the pseudotracheae. When the cords are strongly in action the latter is, however, thrown into folds (Text-fig. 35).

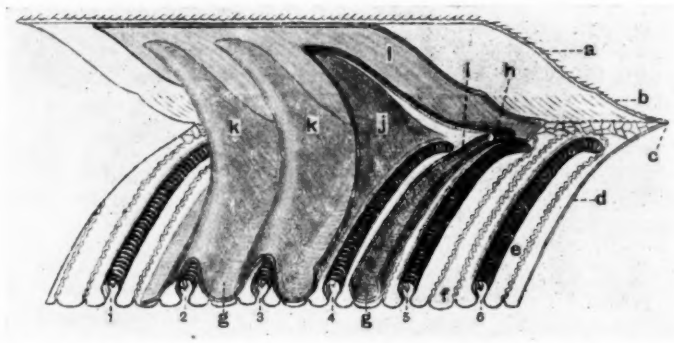
The cords can be traced with ease in many fixed preparations, but are difficult to demonstrate after treatment with potash. In suitably prepared and stained specimens it may be seen that reflections of the hypoderm lining the internal surfaces of the labellum surround the individual cords, leaving elongated spaces between them through which branches of the labial nerve and trachea pass.

(2) *The sub-pseudotracheal membrane.*

A hypodermis of cubical cells underlies the cuticle of the outer wall of the labellum, but the hypodermis underlying the inner wall, while closely attached to the interpseudotracheal plates, is thrown into deep folds under the pseudotracheal plates. In specimens preserved in formalin or alcohol the features of the *sub-pseudotracheal membrane* (G. 2), as the hypodermis underlying the pseudotracheal membrane may be termed, can be studied, either by removing the outer wall of the labellum and thus examining the ab-oral surface of the membrane, or by dissecting away the pseudotracheal membrane from its oral surface, a more difficult operation. With practice this membrane can be dissected out entire, but its relations to the surrounding structures are then difficult to visualise.

In dissections from the ab-oral surface the membrane may be traced from the discal sclerite distally. In the region of the prestomal teeth it underlies their fangs, to whose surfaces it is moulded, and separates them from the labial salivary gland. In the pseudotracheal region the membrane is closely applied to the interpseudotracheal plates, especially at the syndesmoses, but is loose behind the pseudotracheae, resulting in the formation of a series of parallel, *sub-pseudotracheal folds* (G. 2) across the labellum. Passing outwards each sub-pseudotracheal fold becomes progressively looser and deeper. Some distance from the prestomal margin the apices of the folds cease to follow the lines of the pseudotracheae and bend posteriorly, namely, in the opposite

direction to the curved ends of the pseudotracheae, forming a series of *falciform folds* (G. 2) which pass outwards and (in such preparations) upwards to fuse with the hypodermis of the outer wall of the labellum, while the deeper portions of the folds follow the margins of the interpseudotracheal plates. Slender fibrils of chitin pass from the deep surface of each pseudotrachea to the apex of its corresponding fold. Between the falciform folds, but hidden



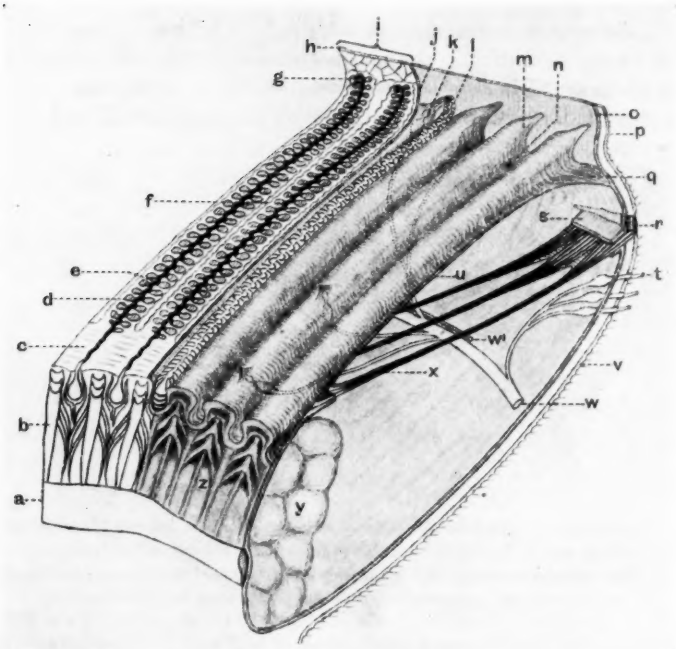
Text-fig. 30. A diagrammatic representation of a dissection of the interior of the labellum near the prestomal margin, to show the falciform folds of the sub-pseudotracheal membrane.

Around pseudotracheae 1 and 6 the sub-pseudotracheal membrane has been removed and the pseudotracheal and interpseudotracheal plates have been exposed. Around pseudotracheae 2 and 3 the sub-pseudotracheal membrane has been left undisturbed, and its pseudotracheal folds are seen developing distally into deep falciform folds (*k*), which pass obliquely to the left to fuse with the hypodermis (*l*) of the external wall of the labellum (*a*). On the left side of pseudotrachea 4 the fold has been cut along its summit leaving its left side in position. Its right side has been cut off at a lower level to show the relationship of the fold to the pseudotrachea. Between pseudotracheae 4 and 5 only the portion of the sub-pseudotracheal membrane which is attached to the interpseudotracheal plate has been left in position in order to show how the long, deep "pseudotracheal pockets," which lie between adjacent pseudotracheae, are formed.

a. Cut edge of the outer wall of the labellum. (For some distance the hypodermis has been removed.) *b*. Marginal strip. *c*. Prestomal margin. *d*. Cut edge of inner wall of labellum (pseudotracheal membrane). *e*. Pseudotracheal plate. *f*. Interpseudotracheal plate. *g*. Part of the sub-pseudotracheal membrane attached to an interpseudotracheal plate and terminating distally in a pseudotracheal pocket (*h*). *i*. Cut edge of the right side of the falciform fold covering pseudotrachea 4. *j*. Inner surface of the left side of the falciform fold covering pseudotrachea 4. *k*. Falciform folds. *l*. Hypodermis covering the outer wall of the labellum.

by them, are deep, oblique *pseudotracheal pockets* (G. 2) leading to the tips of the pseudotracheae and accommodating the *terminal* gustatory sense organs.

If the pseudotracheal membrane be removed and the sub-pseudotracheal membrane viewed from the oral surface (Text-fig. 31), the areas attached to the interpseudotracheal plates appear as ridges, bearing one to four pointed projections, where the gustatory papillae reach the surface. The ridges terminate in larger, nipple-like extremities, which are the oral surfaces of the pseudotracheal pockets between the falciform folds. The loose folds underlying



Text-fig. 31. Diagrammatic representation of a dissection of part of the labellum from the pseudotracheal surface.

a. Bar of discal sclerite. *b*. Dental area of the pseudotracheal membrane with prestomal teeth and the openings of two collecting channels of the central set of pseudotracheae. *c*. Flexural area of the pseudotracheal membrane. *d*. An interbifid groove. *e*. Longitudinal zigzag fissure. *f*. Interpseudotracheal plate. *g*. "Terminal" gustatory papilla. *h*. Prestomal margin. *i*. Intact area of pseudotracheal membrane, supporting two pseudotracheae. *j*. Cut edge of pseudotracheal membrane. To the right of this line the inner chitinous wall of the labellum (pseudotracheal membrane) has been removed to expose the sub-pseudotracheal membrane. *k*. End-organ of the "terminal" gustatory papilla. *l*. Pseudotracheal rings lying in a fold in the sub-pseudotracheal membrane (after the removal of the remainder of the pseudotracheal plate. *m*. Pseudotracheal pocket. *n*. Fold of the sub-pseudotracheal membrane underlying a pseudotrachea. *o*. Cut edge of hypodermis underlying the outer wall of the labellum. *p*. Marginal strip. *q*. Falciform fold. *r*. Epifurca. *s*. Reflections of the hypodermis (cut off close to their origins) covering the tendinous cords. *t*. Macrochaetae of the marginal fold supplied by a branch of the labial nerve. *u*. Cut edge of the sub-pseudotracheal membrane. *v*. Cut edge of the outer chitinous wall of the labellum. *w*. Labial nerve, with branches passing to the macrochaetae (*t*) and gustatory papillae, accompanied by a tracheal trunk (*w'*). *x*. Tendinous cords, with reflections (cut near their origins) of the sub-pseudotracheal membrane covering them. *y*. Labial salivary gland. *z*. Dental area of the sub-pseudotracheal membrane, showing impressions made by the prestomal teeth (which, with their supporting chitinous dental membrane, have been removed).

the pseudotracheae appear as gradually deepening grooves which terminate in deep, oblique, blind sinuses, which are the cavities bounded by the walls of the falciform folds. In the region of the prestomal teeth the moulding of the membrane to the fangs is very noticeable in fixed preparations.

The cells of the sub-pseudotracheal membrane are flattened with rounded nuclei where it underlies the interpseudotracheal plates, but the cells of the sub-pseudotracheal, and especially of the falciform, folds are elongated and contain long, oval nuclei, so that they resemble smooth muscle fibres[1].

The falciform fold seems to exert oblique traction, either actively, if the cells are contractile, or passively, when blood is tending to distend the whole labellum, on the penultimate portion of its corresponding pseudotrachea causing the prestomal margin and the neighbouring region, containing the curved end of the pseudotrachea, to be erected in relation to the rest of the pseudotracheal surface. The "rim," which the fly is apparently able to erect in the "cupping" position, seems to be produced in this way (Text-fig. 36, IV). In preserved specimens it is not uncommon to find erection of one portion only of the rim. In living flies, slight, independent movements of the prestomal margins and neighbouring parts may be seen, probably due to the action of the falciform folds.

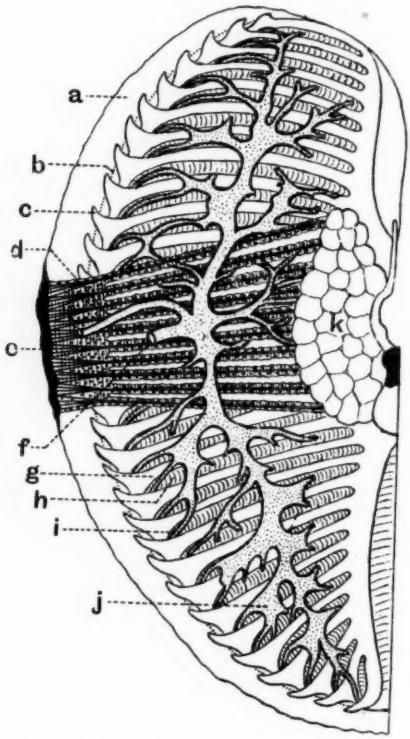
(3) *The distribution of the labial nerve in the labellum.*

The labial nerve in company with the trachea supplying the labellum traverses the haustellum lying between the retractors of the furca and paraphysis, and, after entering the labellum between the former and the labial salivary gland, passes forward to reach the centre of the fenestrated aponeurosis formed by the tendinous cords. Here the nerve divides into several branches (Text-figs. 27, 32). One passes (*a*) forward over the edge of the aponeurosis and lying on the ab-oral surface of the sub-pseudotracheal membrane supplies the anterior portion of the pseudotracheal region, another (*b*) similar branch passes backwards to supply the posterior region, while others (*c*) pass through the aponeurosis to supply the region underlying it. One large branch (*d*), or in some specimens several branches, passes outwards to supply the macrochaetae in the lateral fold, and smaller branches pass to (*e*) the outer wall of the labellum and (*f*) the labial salivary gland.

The nerve and its branches are surrounded by cells, loaded with orange-coloured pigment, which spread out to form an irregular sheet most heavily pigmented and strongest between diverging branches of the nerve. This sheet overlies and is attached to the folds on the ab-oral surface of the sub-pseudotracheal membrane, connecting and perhaps keeping in position their free borders. It is very thin and difficult to dissect, and though Lowne

[1] Kraepelin (1883) admits muscle fibres are absent in the lips, except a few transverse bands near their anterior border. Lowne (1892, p. 399) failed to recognise these, though he says that "fibrous, probably elastic, bands of tissue are present." These authors are possibly referring to the falciform folds.

(1870, p. 49) seems to regard it as a "continuous membrane," it appears to be in many places discontinuous. For descriptive purposes it may be termed the

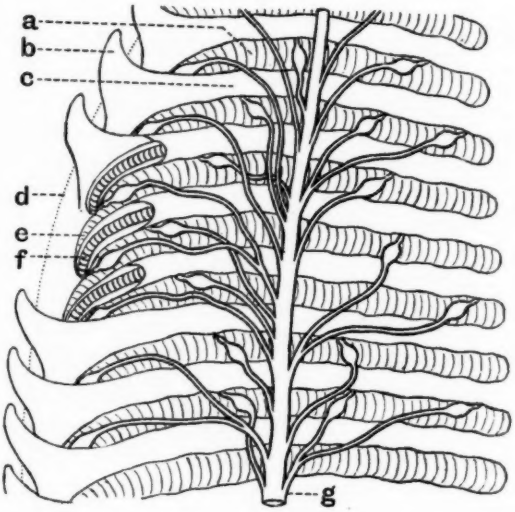


Text-fig. 32. View of the interior of the left labellum after the removal of its outer wall, showing the distribution of the labial nerve and of the *pigmented membrane* (stippled) in connection with it. The central branches of the nerve pass deeply between the tendinous cords, and the anterior and posterior branches lie on the sub-pseudotracheal membrane, sending twigs to the gustatory papillae, etc. In young living specimens the pigment may be seen through the inner wall of the labellum.

a. Portion of the outer wall of the labellum reflected outwards. *b*. Line of prestomal margin (dotted). *c*. Falciform fold. *d*. Branch of the labial nerve passing outwards to supply the macrochaetae of the marginal fold. *e*. Epifurca, with the tendinous cords, covered by reflections of the sub-pseudotracheal membrane, passing inwards from it. *f*. Trunk of labia nerve (cut across). *g*. Fold of sub-pseudotracheal membrane underlying a pseudotrachea. *h*. Depressed portion of the sub-pseudotracheal membrane attached to an interpseudotracheal plate. *i*. A branch of the nerve to a terminal gustatory papilla. *j*. Pigmented membrane (stippled) spreading over the branches of the labial nerve. *k*. Labial salivary gland.

pigmented membrane (G. 2). In the freshly emerged living fly the distribution of the pigment beneath the inner surface of the labellum can be seen through the semi-transparent pseudotracheal membrane.

Text-fig. 32 represents the pigmented areas and the distribution of the labial nerve as seen by removing the outer wall of the labellum in a fresh specimen. Heavily pigmented cells also occur beneath the outer wall of the labellum, especially where the numerous sub-branches of the nerve pass to the end-organs of the sensory macrochaetae in the lateral fold. (Text-fig. 34.)



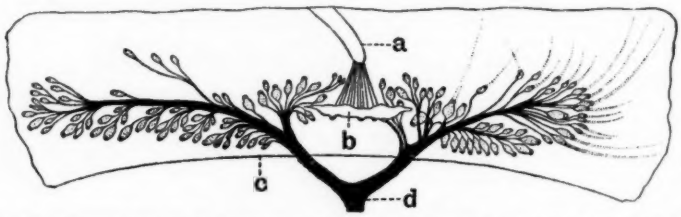
Text-fig. 33. A portion of the internal surface of the sub-pseudotracheal membrane (from a specimen preserved in alcohol in which the pigment has disappeared) to show the distribution of the filaments of the labial nerve to the gustatory papillae.

a. Depressed portion of the sub-pseudotracheal membrane attached to an interpseudotracheal plate. *b*. Falciform fold. *c*. Fold of the sub-pseudotracheal membrane underlying a pseudotrachea. *d*. Line of prestomal margin (dotted). *e*. Pseudotracheal pocket, exposed by removing the overlying falciform fold. *f*. Pseudotrachea, exposed by the removal of the falciform fold above it, with "terminal" gustatory papillae opening at its extremity. *g*. Anterior labellar branch of the labial nerve with filaments supplying the gustatory papillae. The end-organs of these papillae are depicted as swellings.

In certain fixed preparations the pigment tends to disappear and it is possible to follow with greater ease the distribution of the branches of the nerve. From the principal branches *a*, *b* and *c*, fibrils arise, most of which terminate in "gustatory papillae," only very minute fibrils passing into the subpseudotracheal membrane (Text-fig. 33). On nearing the sense-organ the fibril loses its pigmented sheath, and consequently appears to become reduced in diameter, before it enters a pyriform mass of four to six granular cells with large rounded nuclei. (Text-fig. 22.) Beyond the pyriform mass the organ seems to consist of three or four elongated cells, swollen in the neighbourhood of their nuclei, enclosing a minute channel. The tips of the centrally-situated organs project as gustatory papillae (p. 89), through the syndesmoses connecting the

pseudotracheal and interpseudotracheal plates, while tips of the *terminal* gustatory papillae are intimately connected with the distal ends of the pseudotrachea. (Text-fig. 18.)

The branch, *d*, passes towards the epifurca and divides beneath it into two sub-branches, supplying fibrils to the sensory bulbs of the macrochaetae ("gustatory setae," Hewitt) in the lateral fold. In some cases the branch *d* is represented by two or more small branches arising directly from the main stem. "Each of the large marginal sensory bristles of the ab-oral surface has a fine channel running down the whole length of the seta" (Hewitt, p. 61). "Each end-organ consists of a very thin capsule of flat cells, loaded with dark-orange pigment granules. These cells are a part of the hypoderm, which is also similarly pigmented in the neighbourhood of the setae. Within the pigmented cells is a layer of eight or ten fusiform nerve-cells, in which the nerve-fibres terminate; these enclose a single large cell, the trichogenic cell, which



Text-fig. 34. Internal surface of the outer wall of the labellum in the region of the marginal fold showing the distribution of the branch of the labial nerve supplying the sense organs of the macrochaetae. The positions of some of the macrochaetae, which project from the outer surface, are indicated on the right by dotted lines.

a. Terminal portion of the lateral process of the furca. *b*. Epifurca (the macrochaetae arising from it have been omitted). *c*. Line of prestomal margin. *d*. Branch of the labial nerve.

extends into the seta. The trichogenic cell is clear and transparent, or possibly filled with fluid; the nucleus is pressed to one side of the cell by what appears to be a large vacuole. The sheath of the nerve is continued as a thin membrane between the pigmented sheath and the nervous elements, and is apparently attached to the base of the seta" (Lowne, 1892, p. 403). (Text-fig. 22.)

Whatever their function the importance of these bristles is evident from their nerve supply.

(4) *The trachea of the labellum.*

The trachea accompanying the labial nerve divides, like the nerve, into many twigs, which accompany all the branches of the nerve. Lowne figures (1892, p. 394, fig. 50, 1) the folds of the sub-pseudotracheal membrane underlying the pseudotracheae and considers the spaces between the pseudotracheae and the folds to be "air channels," which have the same distribution as the pseudotracheae which they accompany" (p. 398), but no evidence in support of this unlikely view has been obtained.

(5) *The labial salivary glands.*

The labial salivary glands of either side lie at the base of the labellum, limited distally by the insertion of the tendinous cords into the pseudotracheal membrane, and internally by the dental portion of the sub-pseudotracheal membrane underlying the prestomal teeth and bar of the discal sclerite. They extend proximally a short distance into the haustellum at the sides of the labial gutter, and posteriorly are closely connected with the free process of the elastic ligament. Externally they project into the blood space of the labellum (Text-fig. 27). They are composed of large cells somewhat triangular in shape. In *M. domestica*, according to Hewitt (p. 63), "each gland cell is 40μ in size, and possesses a large nucleus (12μ), and internal to this is a permanent circular vacuole which is 16μ in size, and is lined by a thin chitinous intima. The duct of each gland cell opens into the side of the vacuole. The ducts are intracellular, and run from the centre of the gland, some of them uniting, to form a number of fine ducts on the ventral sides of the discal sclerites, which unite and open into the oral pits by a median pair of pores."

XII. THE MOVEMENTS OF THE LABELLA.

The mechanism by which flexion and extension of the oral disc is brought about has been described (p. 79), and some references to the resting and feeding positions of the labella have been made. In this section the means by which the latter positions are attained are described in greater detail.

When the oral disc is extended, but the prestomum still remains closed, the retractor muscles of the furca are relaxed, and the inner surfaces of the labella from the prestomal margins to the central collecting channels are in apposition. Proximally the dental flexures of the central collecting channels separate the surfaces and allow space for the accommodation of the prestomal teeth. In the regions of the common anterior and posterior collecting channels the pseudotracheal surfaces are in apposition throughout. The tendinous cords are lax, the two lateral processes of the furca parallel, the mento-furcal bars lie mainly in the fossae of the lateral processes, the forks of the mentum, mento-furcal bars and lateral processes are in line, and the central spring-like part of the furca is convex proximally.

To produce the *filtering position* (G. 2) the retractor muscles, acting through the epifurcas, draw the lateral processes outwards and (anatomically) upwards, until the pseudotracheal surfaces of the two labella are brought into one plane. During this process the tendinous cords remain lax and do not influence the dental flexures, so that the teeth are not exposed and the pseudotracheal surfaces meet along a slightly depressed line, distal to the commencement of the central collecting channels. This longitudinal depression is clearly visible in impressions of the disc produced in feeding experiments, and may be termed the *prestomal sulcus* (G. 2) (Pl. XX, figs. 15, 16, and fig. 36, III, IV). Simultaneous injection of the blood space with fluid converts the exposed pseudo-

tracheal surface of the disc into a pad which can accommodate itself to irregularities in any surface to which it is applied. The amount of blood injected into the labellum is small and the labella seldom become so distended as they are when pressure is applied to the head in order to distend them. Such specimens present abnormal appearances (Pl. XX, fig. 1). Impressions of the disc in the filtering position are shown in Pl. XX, figs. 15, 16, 17.

The mento-furcal bars still lie in their fossae in line with the lateral processes, but are abducted on the forks at the superior mento-furcal joints, and the convexity of the "spring" is flattened. Accommodation to very irregular surfaces is greatly facilitated by the fact that the two lateral processes of the furca can be moved independently, at any rate to a limited extent.

Text-fig. 35. Diagrammatic longitudinal sections of the oral disc illustrating the attitudes assumed by the labella in the resting and feeding positions and the controlling mechanism. The diagrams show the positions with the disc directed upwards, the reverse of the anatomical position in which it is usually described.

All the feeding positions are consequent on varying degrees of contraction of the retractor muscles of the furca, which draw the lateral processes from the resting position in which they project forwards to the "direct feeding position," in which they lie along the sides of the haustellum pointing backwards. The movements of the lateral processes are transmitted to the distal parts of the pseudotracheal membrane through the epifurca and its strap-like connection in the "filtering" and "cupping positions," and to the proximal portions of the membrane through the tendinous cords passing from the epifurca to the flexural area in the "intermediate," "scraping" and "direct-feeding" positions.

I. The *resting position*. In this position the prestomal margins and the inner surfaces of the pseudotracheal membranes are in apposition. To avoid complication they are shown as slightly separated.

II. The *filtering position*. The pseudotracheal membranes are still in apposition immediately above the dental flexures, but their distal portions have been drawn apart by the traction of the lateral processes of the furca, through the epifurcas, on the marginal folds. The tendinous cords have not been influenced.

III. The *cupping position*. The "rims" of the labella have been erected, but the relationships of the other structures have undergone no appreciable alteration.

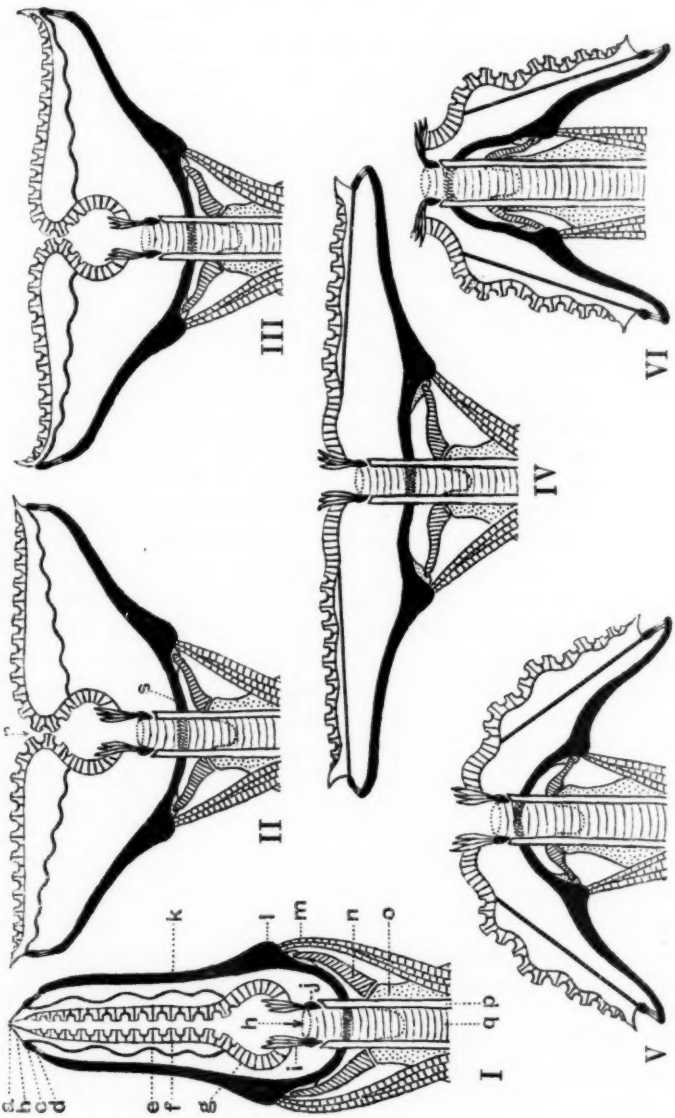
IV. The *intermediate position*. Further separation of the lateral processes has caused the tendinous cords to become taut and draw aside the dental flexures sufficiently to expose the teeth.

V. The *scraping position*. Further contraction of the retractor muscles has caused the lateral processes to be drawn backwards over the distal part of the haustellum, carrying with them the pseudotracheal membranes, which are thrown into folds. The traction exerted by the tendinous cords on the flexural and dental areas has fully exposed and separated the prestomal teeth.

VI. The *direct-feeding position*. This is an exaggeration of the scraping position in which further traction by the tendinous cords has caused even the teeth to be everted. The "oral aperture" is completely exposed.

a. Prestomal margin. *b*. Marginal strip. *c*. Epifurca. *d*. Strap-like connection between the epifurca and the lateral process of the furca. *e*. Tendinous cord. *f*. Pseudotrachea membrane. *g*. Dental flexure. *h*. Oral aperture (dotted). *i*. Prestomal teeth. *j*. Discal sclerite (in section). *k*. Lateral process of furca. *l*. Tubercle of lateral process of furca. *m*. Retractor muscle of furca.) *n*. Mento-furcal bar. (Its distal extremity lodges in a fossa (stippled) in the lateral process of the furca.) *o*. Mentum. *p*. Paraphysis. *q*. Hypoglossa. *r*. Prestomal sulcus. *s*. "Spring" of the furca, shaded where it passes behind the hypoglossa. (Its central process has been omitted.)

Conversion of the "filtering position" into the *cupping position* (G. 2) is brought about by the erection of the "rim," namely the marginal strip, by



Text-fig. 36.

the action either of the falciform folds or by increased pressure of blood, without appreciable alteration in the relationship of the other structures to

each other, though the pseudotracheal membrane may sometimes be thrown into broad folds. The disc now resembles a Büchner's funnel.

Moulds of the disc in this position are shown in Pl. XXI, figs. 4, 5, 6.

While in the "filtering position" the tips of the prestomal teeth may be exposed by further contraction of the retractor muscles causing sufficient tension on the tendinous cords to draw aside the dental flexures. This may be termed the *intermediate position* (G. 2).

Impressions of the disc in this position are shown in Pl. XXI, figs. 1, 2, 3.

Further contraction of the retractor muscles causes the lateral processes to be drawn further back over the distal end of the haustellum, resulting in the pseudotracheal membranes being carried beyond the horizontal. The traction is communicated to the tendinous cords, which become taut and draw the dental flexures outwards so that the prestomal teeth are separated and fully exposed. (Text-fig. 36, I.) At the same time blood is withdrawn from the labella and the pseudotracheal membranes are thrown into folds. This is the *scraping position* (G. 2) in which the filtering apparatus is out of action, and the exposed teeth are so stabilised by the tension on the membranes supporting their fangs that it is difficult in an anaesthetised specimen to move them in any direction by strong pressure with a needle. Slight momentary application of the proboscis (by the fly) to a suitable surface leaves an impression showing three nearly parallel rows of incised punctures (Pl. XXI, fig. 7), but the antero-posterior cutting motion usually employed by the fly produces a network of short scars and ultimately a pit with a scarred base (Pl. XXI, figs. 8, 9, 10, 11).

The distal portions of the mento-furcal bars still lie in their fossae, but the superior mento-furcal joints are strongly abducted, and "spring" of the furca becomes concave proximally. (Text-fig. 35, V.)

The *direct feeding position* (G. 2) is an exaggeration of the scraping position, the lateral processes being drawn back against the sides of the haustellum and the pseudotracheal surfaces completely everted. Such traction is exerted by the tendinous cords that the lateral plates of the discal sclerite and the prestomal teeth are brought into the horizontal position, and the oral aperture is completely exposed (Text-figs. 35, VI; 36, II).

In this position neither the filtering apparatus nor the prestomal teeth can function, and all objects smaller than the oral aperture are ingested.

Alterations in the relative positions of the mentum and discal sclerite accompany these changes in feeding positions. The central process of the furca, supporting the neighbourhood of the common posterior collecting channels, changes its shape according to the position of the "spring" from which it arises. Anteriorly the cochleariform processes and "brushes," supporting the neighbourhood of the common anterior collecting channels, remain in apposition throughout and prevent leakage through the dorsal seam.

The return of the labella to the parallel resting position after relaxation of the retractor muscles is brought about by the recoil of several chitinous structures, (*a*) the "spring" of the furca, (*b*) the lateral plates of the discal

sclerites, and (*c*) the dental flexures. In the approximation of the labella the distal regions of the dental flexures are the first parts to meet.

XIII. FEEDING EXPERIMENTS.

Feeding experiments of various kinds were carried out in order to test the suggestions derived from the study of dissections.

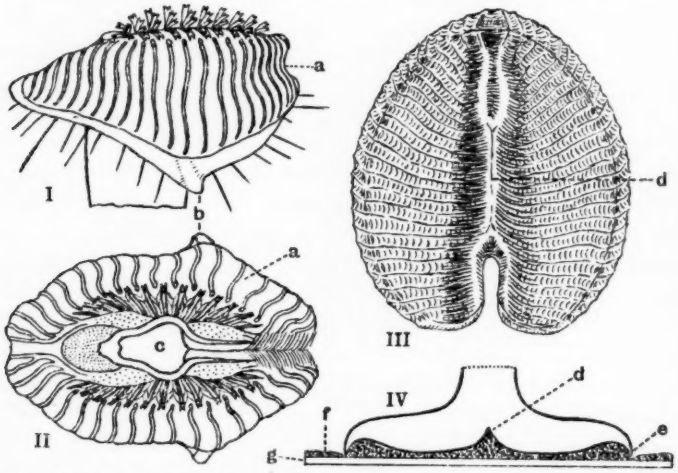
In one series flies were allowed to feed on thin, drying films of sugar solution, containing finely ground Indian ink, deposited on glass. In such experiments the "filtering position" is almost always assumed, and the proboscides are so closely applied that the zigzag fissures of the pseudotracheae are closed and to a great extent the prestomal sulcus is obliterated. Under these conditions food cannot enter the oral aperture except through the interbifid grooves. A fly which is allowed to suck, and forced to move on as soon as it lifts its proboscis in order to prevent the imprints from becoming superimposed, leaves beautiful isolated impressions of the applied portions of the disc (Pl. XX, fig. 15). The outline is oval, corresponding to that of the pseudotracheal area which is exposed when slight pressure is made on the head of the anaesthetised fly (Pl. XX, fig. 14), and the line of the almost obliterated prestomal sulcus and the region occupied by the interlabellar membrane are clearly visible. The longitudinal axis of each pseudotrachea is marked by a zigzag black line, running outwards from the prestomal sulcus, which represents the area covered by the apposed sides of the longitudinal fissure. Of the zigzag longitudinal fissure itself no trace can be seen. On each side of the zigzag line is a row of regularly placed, clear, oval areas. Each of these areas, from which the pigment has been removed by suction, represents a space covered by an interbifid groove, and the thin black lines separating them represent the lateral walls of the grooves. The broader black band, which separates the marks left by one pseudotrachea and its interbifid grooves from the marks left by the next, represents an interpseudotracheal plate. These bands usually become broader towards the periphery, and vary in width in different impressions, depending on the degree of blood pressure within the labellum (Pl. XX, figs. 15, 16, 17). The way in which the pattern is produced is best understood by reference to Text-figs. 17, 18, 21, 31.

Exactly similar impressions are left in films of drying milk.

In another series flies were allowed to feed on deeper films of similar fluids.

In a film of suitable depth the suction sets up currents, which draw particles of ink towards the disc from all points, leaving a moderaly clear zone round it (Pl. XX, fig. 13). Depending on the depth of the film and the concentration of the pigment impressions of different kinds are left. (*a*) When shallow films containing little Indian ink are employed the marks are heart-shaped, clear in the centre and outlined by a zone of pigment. Probably the "cupping position" has been used, and the pigment drawn towards the disc arrested at the "rim." (*b*) When deeper films containing much Indian ink are used, the disc, in the "cupping position," is not pressed down on to the

glass, but the fluid together with the smaller particles is sucked through the interbifid grooves and the larger particles of the pigment collect on the pseudotracheal surfaces and are rejected. In this way very beautiful moulds of the disc are left (Pl. XXI, figs. 4, 5, 6). In most of these there is a central, longitudinal **V**-shaped elevation, representing the prestomal sulcus, which occasionally exhibits bifid terminations, indicating the common anterior and posterior collecting channels. The oval external margin seems to mark the



Text-fig. 36. I. Side view of oral disc in the "scraping position." II. Distal view of oral disc in the "direct-feeding position" to show the labella and teeth strongly everted so as to expose the oral aperture completely. III. Mould of the oral disc in the "cupping-position," showing impressions of the prestomal sulcus, anterior and posterior collecting channels and pseudotracheae. IV. Diagrammatic transverse section across the disc in the act of producing such a mould.

a. Everted labellum. *b*. Distal extremity of the lateral process of the furca. *c*. Oral aperture. *d*. Prestomal sulcus. *e*. "Rim" of labellum. *f*. Layer of Indian ink and syrup. *g*. Glass plate.

outline of the erected "rim," and within this there is usually an elevated portion, corresponding to the hollow in the pseudotracheal surface internal to the erected "rim" (Text-fig. 37, III, IV). Moulds of the pseudotracheae may, or may not, be present, but when well-preserved they extend over the elevated portion to the margin (Pl. XXI, fig. 6). Sometimes large clear spaces are present on each side of the median elevation, showing that the corresponding areas of the pseudotracheal membrane have been pressed against the glass.

In order to obtain some information on the use of the teeth, flies were allowed to walk over films of semi-solid sugar-agar or other suitable substances[1].

[1] Suffolk (1869) wrote as follows: "The office of the teeth is undoubtedly that of cutting instruments or scrapers. I have examined carraway comfits after flies have fed upon them and found them covered with small parallel scratches, the distance of which, when measured with the micrometer, corresponded exactly with that between the teeth." Lowne (1870, p. 48) says that the teeth "are used when exposed for grinding hard substances such as sugar."

If unable to obtain sufficient moisture from the surface flies use their teeth to scrape or excavate the agar. The marks left on the agar depend on the extent to which the teeth are exposed and on whether movements designed for cutting occur or not. If the disc is applied in the "intermediate position," a longitudinal line of punctures or scars, or occasionally an elongated pit, in the centre of the oval labellar impression, is produced (Pl. XXI, figs. 1, 2, 3). When the disc is applied momentarily in the "scraping position" with the teeth fully exposed six rows of punctures are produced, three on each side of a median unscarred area, representing the "oral aperture," converging towards the ends of the pattern (Pl. XXI, fig. 7). When in the "scraping position" forward and backward cutting movements of the proboscis occur, the surface of the agar is torn by a number of small incisions over a limited area, or if the movements are continued a deep cavity with a scarred base may be excavated (Pl. XXI, figs. 8, 9, 10, 11).

When soft agar is employed the fly often attempts to suck it up without the intervention of the filtering apparatus by thrusting its proboscis in the "direct-feeding position" below the surface. Oval excavations with pointed ends are produced, which exhibit on their sides impressions of the bases of the everted teeth and of the mouths of the collecting channels (Pl. XXI, fig. 12).

It seems from the small series of experiments that have been carried out with different food materials that blow-flies use the "filtering position" when feeding on thin films, but the "cupping position" when feeding on drops or moderately deep layers of liquids, such as syrup, milk, serum or pus. When feeding on tenacious or semi-solid materials, such as sputum or faeces, they use the "direct-feeding" position (Pl. XXII). They have been seen to "scrape" such objects as sugar, recent blood clots and the surfaces of healing wounds. After scraping the surface is moistened with "vomit" or saliva and then suction is made with the disc applied in the filtering position.

XIV. THE REGURGITATION OF FOOD.

After a meal food is "regurgitated into the mouth and prestomum, or even forms a drop between the partially-closed lobes of the oral sucker before it is finally swallowed and transmitted by the proventriculus to the chyle stomach, a fact known to and recorded by Reaumur" (1738), (Lowne, p. 402). No valve has been described in the food canal as it passes through the proboscis but Lowne (1870, p. 42) thinks that the peculiar action of the dilator muscle of the pharynx accounts for the well-known phenomenon of regurgitation and reswallowing of liquid food. "The dilator of this cavity draws fluid from the mouth and its obliteration, which is entirely effected by the elasticity of its walls, injects it into the oesophagus and crop, the absence of valves being made up for by the peculiar rhythmic action of its muscles, the anterior fibres contracting and again relaxing before the posterior, so that an undulating motion is given to the superior wall of the cavity." He states later (1892, p. 390) that

"the undulating movement is capable of effecting a flow of fluid either from or towards the mouth."

Apart from the action attributed to the dilator pharyngis muscle by Lowne it is possible that, while the pharynx is open, part of the contents of the crop may be expelled into the prestomum by contraction of the muscle fibres in its walls and withdrawn by their relaxation.

XV. The modes of distributing infection.

After feeding on any material containing pathogenic organisms the crop contents remain infective for some days and the habit of regurgitation ensures that the inner surfaces of the labella are frequently re-infected. By means of its "vomit" or by the application of its oral disc the fly is liable to infect foods or skin or mucous surfaces. The infection of food can also be brought about by the deposition of faeces upon it. Several observers have shown that the ova of helminths, ingested with human excreta, are expelled in the faeces of the fly, but they do not appear to be regurgitated in the "vomit." The prestomal teeth, moistened with infected vomit, appear to be most efficient instruments for the intradermal introduction of pathogenic organisms. The sites on the body most likely to be chosen for scraping are the mucous and conjunctival surfaces and recent abrasions.

XVI. Summary.

A detailed description of the anatomy of the proboscis of the blow-fly, *C. erythrocephala*, and of the functions of the structures found in it is given. Attention is particularly directed to the fact that the fly can feed in several ways. It can filter the larger particles out of its liquid food, or can scrape chosen surfaces, moistened with vomit or saliva, with its prestomal teeth and subsequently ingest the emulsion produced, or can suck up materials, such as thick sputum or faeces containing helminth ova, without the intervention of its filtering apparatus or the application of its teeth. The relation of these modes of feeding to infection is very briefly indicated.

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DESCRIPTION OF PLATES XX—XXII.

PLATE XX.

Figs. 1–11 and 14 are from specimens fixed in absolute alcohol, treated with alcoholic sodium hydrate solution, cleared and mounted in cells without pressure.

Fig. 1. Proboscis from the dorsal surface to show the relationship of the chitinous structures. The labella are abnormally distended, since the organ was erected before fixation by pressure on the head.

Fig. 2. Side view of the proboscis in the "resting position," with the disc flexed on the haustellum.

Fig. 3. Side view of the extended proboscis. The disc is extended on the haustellum and the forks of the mentum, mento-furcal bars and lateral processes of the furca are in line, but the labella are still in apposition.

Fig. 4. Side view of the extended proboscis with the labella separated in the "filtering position." The lateral process of the furca is partly flexed on the mentum. The mento-furcal bar connecting these structures is distinguishable.

Fig. 5. Dorsal view of the distal portion of the proboscis in the "direct-feeding" position.

Fig. 6. Ventral view corresponding to Fig. 8. The partially everted teeth are visible above and the arrows point to the distal ends of the strongly flexed lateral processes of the furca projecting on each side.

Fig. 7. Side view in the "scraping position" with the teeth fully exposed. The labella are turned back on the distal end of the haustellum.

Fig. 8. Side view, between the "scraping" and "direct-feeding positions," with the teeth partly everted.

Fig. 9. Side view, near the "direct-feeding position," with the teeth almost completely everted.

Fig. 10. Side view in the "direct-feeding position," with the teeth completely everted. The nodulus (arrow) is seen projecting above in Figs. 9 and 10.

Fig. 11. Dorsal view corresponding to Fig. 9.

Fig. 12. Proboscis marks (natural size) made by flies feeding (filtering position) on a film of partially dried Indian ink and syrup deposited on glass.

Fig. 13. Marks made by a fly walking over and feeding (cupping position) on a moderately thin layer of Indian ink and syrup. Around each mark is a clear zone from which the particles of the ink have been drawn on to the surface of the disc. The larger particles of the pigment which collected on the surface of the disc were rejected and formed moulds of its surface (see Pl. XXI, figs. 4, 5, 6).

Fig. 14. The pseudotracheal surface of the disc in the "filtering position."

Fig. 15. An impression of the disc ($\times 20$) left by a fly feeding in the "filtering position" on a partially dried film of Indian ink and syrup. The outlines of the disc, and the positions of the pseudotracheae and of the prestomal sulcus are well shown.

Fig. 16. A portion of a similar impression more highly magnified ($\times 77$). The lines of the pseudotracheae are marked by double rows of regularly placed clear oval areas, separated by thin zigzag lines. Each of these areas, from which the pigment has been removed by suction, represents the space covered by an interbifid groove. The dark areas between the pseudotracheal impressions represent the interpseudotracheal plates. These are more clearly seen in Fig. 15. In the "filtering position" the two labella diverge at the base of the prestomal sulcus, which is represented by an irregular vertical line.

Fig. 17. Part of a similar impression still more highly magnified ($\times 770$). The longitudinal axis of each pseudotrachea is marked by a black zigzag line, showing that the "longitudinal fissure" was closed. On each side of the black zigzag line are clear oval areas produced by the removal of pigment through the interbifid grooves. Their shapes are clearly defined. The way in which

this pattern is produced can be readily comprehended by reference to Text-fig. 21 (left side). The broader black lines, separating the pseudotracheal impressions, represent the inter-pseudotracheal plates.

PLATE XXI.

Figs. 1, 2, 3. Impressions made by flies feeding in the "intermediate position" on layers of recently set nutrient agar. In all, the outlines of the expanded disc can be seen. In Fig. 1 a number of minute punctures made by the tips of the teeth are visible in the centre, and in Fig. 2 the punctures are deeper and better defined. In Fig. 3 the agar has been scraped through and removed in the area covered by the teeth.

Figs. 4, 5, 6. Moulds of the disc left by flies feeding in the "cupping position" on thick emulsions of coarsely ground Indian ink in syrup spread on glass (see Pl. XX, fig. 13). When the fly ceases to suck closely packed particles of Indian ink, which have been drawn on to the surface of the disc, but owing to their size have failed to pass through the interbifid grooves, are left on the glass in the form of moulds of the disc. In all these moulds the outlines of the disc and the impressions of the prestomal sulcus are clearly defined. In Fig. 5 (left side) the impression of the erected "rim," and in Fig. 6 the impressions of the pseudotracheae are visible.

Fig. 7. Three rows of punctures, on each side of a central clear area representing the oral aperture, made in solidified agar by a fly which momentarily applied its proboscis in the "scraping position."

Figs. 8, 9, 10, 11. Marks made by flies scraping the surface of solidified nutrient agar with their teeth in the "scraping position."

Fig. 12. An impression made by a fly which thrust its proboscis in the "direct-feeding position" into recently set agar. The impressions of the bases of the everted teeth can be seen on each side of the clear central area representing the oral aperture.

Fig. 13. A normal pseudotracheal ring.

Fig. 14. A slightly distorted pseudotracheal ring, showing its bifid and flattened extremities. ($\times$ 700.)

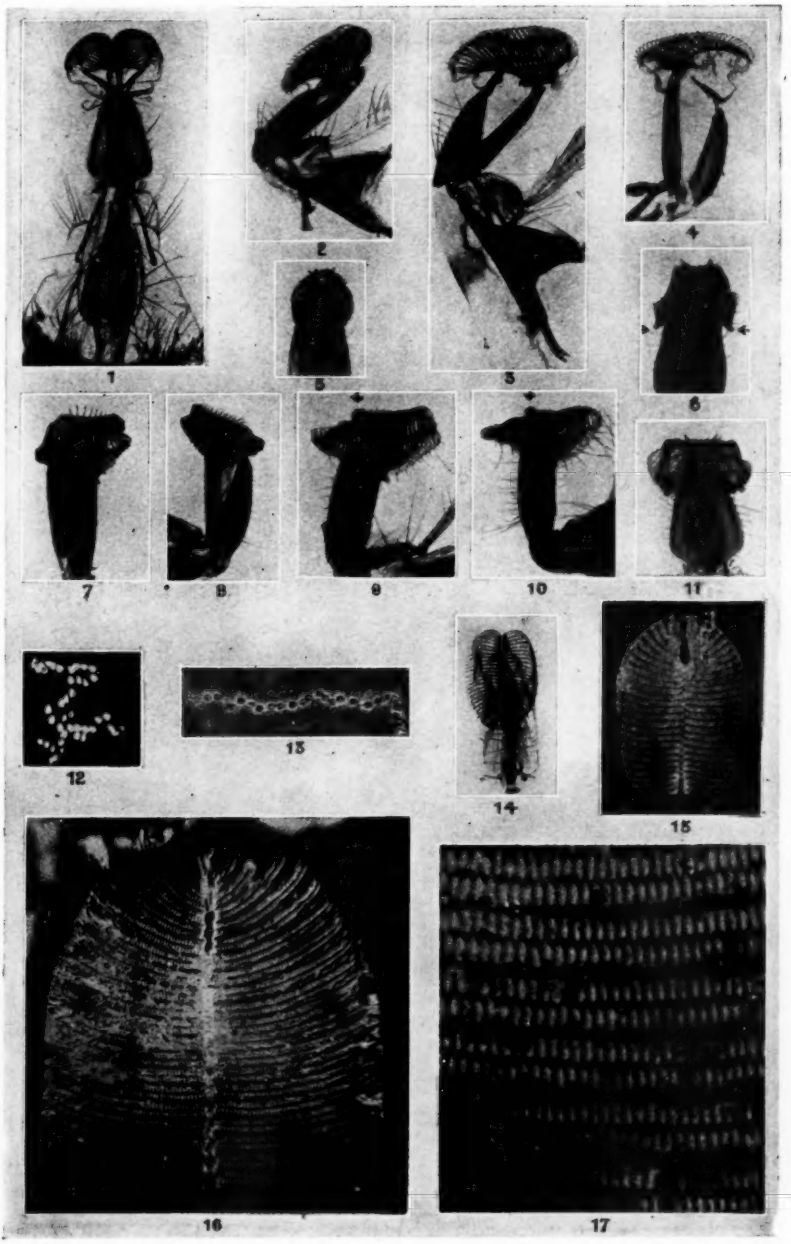
Fig. 15. Part of the pseudotracheal surface of the labellum treated with potash ($\times$340), showing portions of four pseudotracheae. The flattened and bifid extremities of alternate pseudotracheal rings are well seen. Three gustatory papillae are visible.

Fig. 16. Ten consecutive pseudotracheal rings ($\times$700), treated with potash and compressed, showing their alternate flattened and bifid extremities.

Fig. 17. A transverse section ($\times$600) of part of the oral surface of a labellum. Four pseudotracheae are included in the section. In each the ring and the opening of the longitudinal fissure is very distinct. It happens that in each case the bifid extremity is situated on the left side of the pseudotrachea. The point of bifurcation is indicated by a dark spot, above which the forks are curved inwards. From the point of bifurcation a distinct line, which represents the reflection of the cuticle at the base of the interbifid groove, passes obliquely upwards and outwards to the surface of the integument. If the integument is traced from the right side of the longitudinal fissure of the pseudotrachea on the left two deep depressions are seen with a plateau between them. The former are the syndesmoses between the pseudotracheal and interpseudotracheal plates and the latter the interpseudotracheal plate. The sub-pseudotracheal membrane is seen below the pseudotracheae (see also Text-fig. 22, IV).

Fig. 18. A longitudinal section ($\times$600) through a pseudotrachea slightly to one side of the median line. The interbifid spaces and the manner in which the integument passes over and binds together the flattened extremities of the rings and the contiguous forks of the adjacent rings on each side can be seen clearly (see also Text-fig. 22, I).

For these photographs (except Figs. 16 and 17 in Pl. XX and Figs. 13–18 in Pl. XXI, which are reproduced from Graham-Smith (1911), *Journal of Hygiene*), I am indebted to the care and skill of Mr H. P. Hudson, of the Pathological Department, Cambridge.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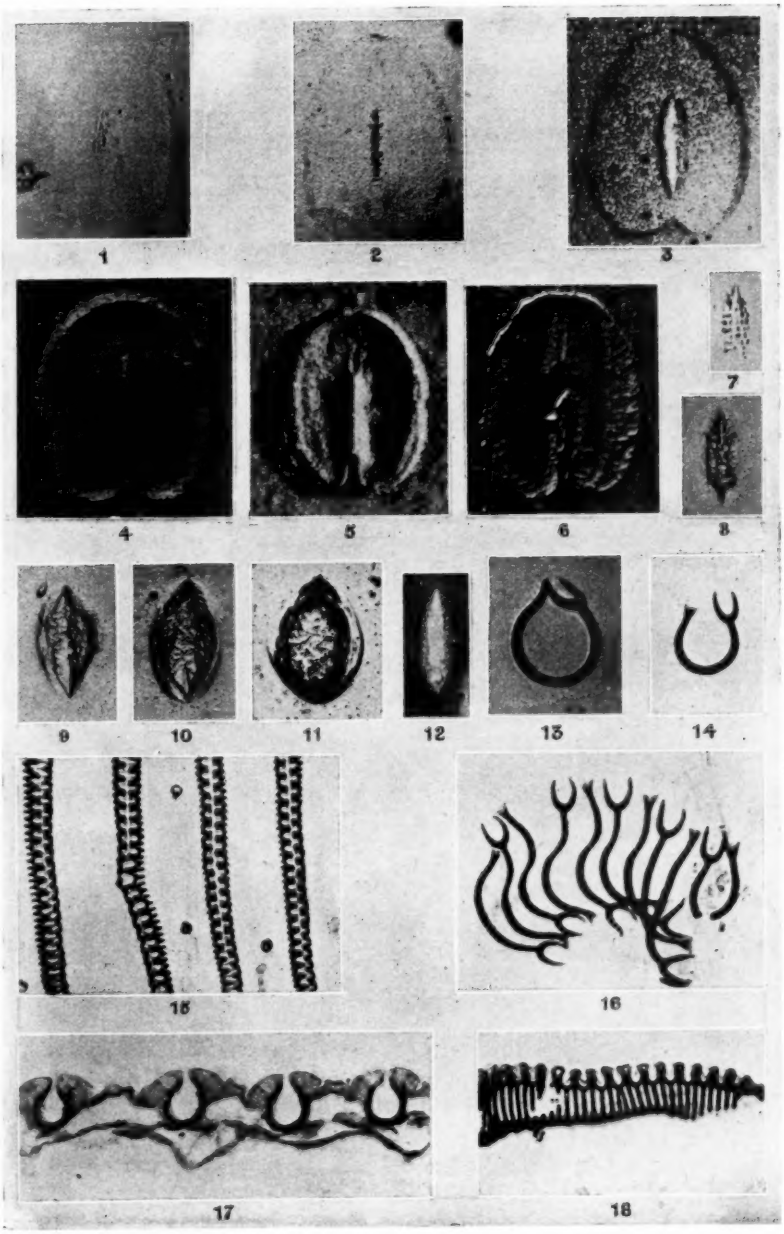
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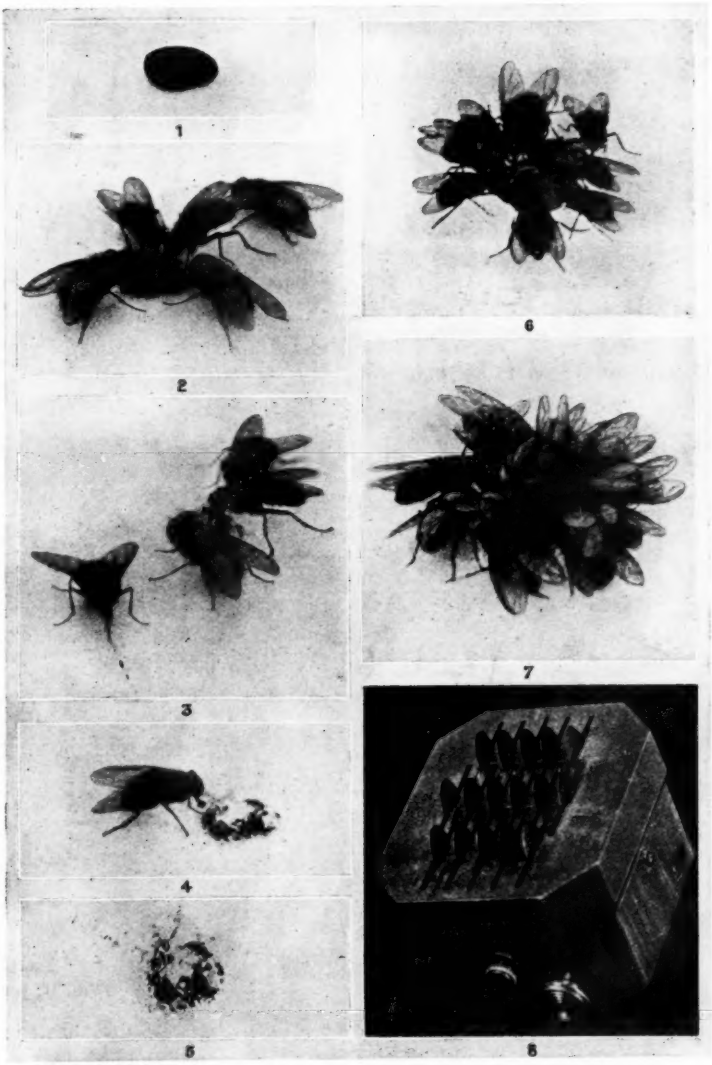

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PLATE XXII.

Fig. 1. A drop of semi-liquid human faeces (natural size).

Fig. 2. Three *C. erythrocephala* and one *Sarcophaga* feeding on the drop, while one *erythrocephala*, having fed, is cleaning itself near by.

Fig. 3. Three flies still feeding, while on the left (*C. erythrocephala*) is cleaning its anterior legs, after depositing two small masses of "vomit," which can be seen in front of it.

Fig. 4. The fly seen on the left of Fig. 3 has returned and is attempting to suck up any semi-liquid material which it can find in the remains of the faeces. The other flies have gone away.

Fig. 5. The dry residue of the drop of faeces, consisting of fragments of vegetable fibres, etc. The drop of faeces was reduced to the condition shown in Fig. 5 in about eight minutes.

Fig. 6. Eleven *C. erythrocephala* feeding on a small mass of human faeces. Some of the flies are standing on the backs of others.

Fig. 7. Fifteen *C. erythrocephala* eagerly feeding on a mass of human faeces.

Fig. 8. A scarificator with its sixteen blades exposed.

(*MS. received for publication* 12. VII. 1929.—Ed.)

ON METHODS OF COUNTING NEMATODE OVA IN SHEEP DUNG.

By NORMAN R. STOLL, Sc.D.

Department of Animal Pathology, Rockefeller Institute for Medical Research, Princeton, N.J.

(With Plate XXIII, and 1 Text-figure.)

The determination of the presence or absence of helminth eggs in the faeces of infected hosts has been an accepted diagnostic method for about half a century. In recent years human parasitology has gone a step further. The fact that the familiar nematodes of the intestinal tract, such as hookworm, *Ascaris* and *Trichuris*, do not multiply in their host but send out through the excreta relatively enormous numbers of eggs, has brought into considerable use the determination of the number of ova passed as an indirect measure of the number of worms harboured. Considering the very large number of helminthic species inhabiting the gastro-intestinal tract of domestic animals, it is probable that in this field also profitable use may be made of egg-count methods in the determination of the severity of infestations.

During the past two years the author has been studying from this angle *Haemonchus contortus*, the common stomach worm in sheep. This has led to the adaptation and adoption of the dilution egg counting and Lane's direct centrifugal flotation techniques as standard methods.

As a preliminary to the publication of the observations on the parasite in question (Stoll, 1929), and because the techniques may be of interest to other workers, it has seemed desirable to report on them in some detail. The latter word is used advisedly for it is the author's observation that success or failure with these methods depends upon a perfection of detail, which in certain respects may be found to vary somewhat with different species of worms and hosts, and that, granted this technical precision, results of a high and known grade of reliability may be achieved.

The two techniques will now be discussed, and analysed as to their routine efficiency, primarily with reference to *Haemonchus* ova in sheep dung.

Dilution egg counting.

This method, described by the author (Stoll, 1923 *a*) for use on human stools, sought, among other items, uniformity of conditions from specimen to specimen, least possible amount of contact with the faeces, establishment of the

count from a fairly large proportion of the original stool, a relatively large amount of mixture for transferring to and counting on the slide, and a degree of dilution usable without change for either soft or firm specimens. In addition it was hoped the diluent used would, at least to a certain extent, deodorise and disinfect the material used. All of these desirable features obtain for the technique as used on sheep dung.

In principle the method is simple: A weighed amount of faeces is comminuted in a known amount of diluent in a flask, in which mixture the parasite eggs become detached from the faecal debris. This mixture, after thorough shaking, is promptly sampled with a pipette with which is withdrawn a known small amount for transfer to a slide, where the ova are counted. The number of ova present per gram of faeces is then determined by multiplying an average slide count by a factor, which is merely the proportion of the original faeces actually present on the slide.

The steps in the method are as follows:

I. Collect an uncontaminated faecal specimen from the sheep.

II. Weigh into a 300 c.c. Erlenmeyer flask (which has been etched at the 300 c.c. level) 10 grm. of the pellets, add decinormal sodium hydroxide to barely cover them, and set aside for a few hours or overnight to soften (in warm weather use the refrigerator).

III. After the pellets are well softened, mash them in the flask with a glass rod which has been flattened at the end. When they are visibly well crushed, add sufficiently more hydroxide to make 300 c.c., put in several solid glass beads, rubber stopper the flask, give it a preliminary shaking and again set aside for a few hours or overnight.

IV. Following thorough shaking, both to complete the freeing of the ova from the faecal debris, and to secure a suspension in which the eggs are well distributed, immediately withdraw into a calibrated pipette 150 c.mm. (0·15 c.c.) of the mixture and transfer to a $1\frac{1}{2} \times 3$ inch slide.

V. Cover the drop with a cover slip of appropriate size, and count the ova in the entire preparation with a microscope possessing a mechanical stage.

VI. Sufficient drops are counted in succession from the flask until the average count deviates less than 5 per cent. from the average secured previous to the last counted drop. This accepted average count multiplied by 200 represents the number of eggs per gram of the faeces originally tubed.

Discussion of the steps will next be given, numbered to correspond to those above.

1. Sheep are usually sufficiently disturbed when herded into a corner of an enclosure to cause them to defecate freely, so that appropriate containers may be used to secure the dung before it reaches the ground[1]. Success may also be had in securing a rectal specimen by hastening defecation with the use of a small glass rod, flame-smoothed at the ends, and dipped in olive or mineral

[1] Paraffined paper containers, ¼ or ½ pint size, manufactured by the Purity Paper Vessels Company, Clipper Mill Road, Baltimore, have proved very serviceable.

oil to prevent abrading the rectal mucous membrane. Veglia (1915) makes use of the finger. Specimens collected from the soil are usually sufficiently contaminated by free-living nematodes and insects to cause difficulties at the microscope later.

Where more complete faecal samples covering a given period are desired the animal may be placed in a clean (helminth-free) crate in which the animal's dung is not trampled by the feet or allowed to remain in the urine. For rams and wethers use may be made of a canvas bag fitting over the rump and held in place by ropes attached to a surcingle around the body just behind the shoulders.

Faecal specimens should be prevented from drying out before tubing, and are preferably refrigerated to prevent development of ova.

2. Pyrex Erlenmeyer flasks of 300 c.c. capacity, taking a No. 6 solid rubber stopper, are standard glassware and when etched at the 300 c.c. level still give a sufficient shaking space to secure effective comminution. The use of 10 grm. of faeces is designed primarily to hold down percentage errors in routine weighing of specimens on the ordinary laboratory balances (Harvard trip scales). The ratio of faeces in total diluent at 1 : 30 is only half as concentrated as the 1 : 15 ratio used on human faeces. This permits utilising sheep pellets without any straining to remove coarse debris or decanting to remove deeply pigmented fluid. While, when carefully performed, such procedures need not cause loss of ova, they introduce additional manipulation which presents that much additional opportunity for error.

Decinormal (or one-tenth molar) sodium hydroxide is approximately a 0·4 per cent. solution (4 grm. of caustic soda made up to one litre with water). As a routine matter it is preferable to have a large stock of normal solution (40 grm. technical NaOH sticks to the litre) from which to make up the decinormal solution.

The caution to place in the refrigerator the flasks containing pellets barely covered with the diluent at this stage arises from the fact that *Haemonchus* ova, like those of hookworms and many other nematodes, go on to embryonation in this dilute sodium hydroxide provided they have sufficient oxygen tension and appropriate temperature.

3. The crushing of the pellets is best done thoroughly, for effective comminution at this stage makes needless inordinate shaking to secure the same result later. Sheep pellets are typically coated with a thin envelope of mucus, but this does not interfere. After the flasks have been filled to the 300 c.c. level, refrigeration to control development of the ova is less important unless under high summer temperatures. Except immediately after shaking, the ova settle to the bottom of the flask where the oxygen tension is too low to permit development. After several days an effect on the ova by the diluent will be noted, causing a granulation and paling of the contents. This does not affect routine counts up to about a week or ten days following tubing. As under usual conditions specimens are egg-counted the second day after tubing

this granulation effect is thus of no consequence[1]. One such egg is visible in Pl. XXIII, fig. 1, at about "11 o'clock."

The solid glass beads we prefer are 5 mm. in diameter.

4. Dilution flask preparations made up as outlined should show little or no floating debris, unless the pellets are very much desiccated before tubing, or unless the shaking has been very vigorous. In the latter case there is a transient float due to debris being upheld by air bubbles caught in the mixture during shaking. We have been able to secure no evidence that such a float selectively deprives the faecal suspension of ova.

It should be emphasised that the shaking process is invoked for two distinct reasons. It is designed in the first place to free the ova from the faecal debris to which they have become adherent during their passage through the gut of the host. The second occasion for shaking is to secure as homogeneous a suspension of the ova in the flask as possible preparatory to withdrawing a drop for counting. While the herbivorous debris in sheep faeces might appear to circumvent the fair sampling of a flask of material for its ova, as a matter of fact the evidence suggests on the contrary that an excellent approximation to a fair distribution of ova is maintained and can be sampled, when the flask is properly handled. At successive samplings of the flask with a pipette, there is the requirement of successive shakings preliminary to them.

The pipettes used may be drawn from glass tubing. Either mercury or the simple procedure earlier described (Stoll, 1923 *a*) may be used to calibrate them[2].

Before withdrawing the drop the mixture in the flask is drawn into and ejected from the pipette a few times, then drawn *quickly* to the marked level and the pipette withdrawn. In transferring the drop to the slide the pipette is held vertically.

The $1\frac{1}{2} \times 3$ inch slides are preferable to 2×3 inch. Unless the drop is divided and small covers used the ordinary 1×3 inch slide is too small.

5. Either a 25 mm. square (1 inch) or 22×30 mm. cover slip is preferred. Occasionally in specimens with unusually deep pigmentation a large cover (22×40 mm.) is advantageous. The use of No. 2 thickness covers cuts down breakage.

In examining the cover-slip preparation it is considered important to habitually follow a uniform procedure, so that if interrupted the person counting may not be confused. Our routine is first to examine the margin outside the cover all around; then to begin always at the upper right or upper left corner of the cover slip (the microscopic field shows it as a lower corner due to the reversal of the image), and using the mechanical stage pass over a

[1] Occasionally animals which are ill and eating or ruminating but little, will have a greatly decreased faecal output, perhaps a tenth or less of the usual daily quantity. Under these conditions, the faeces which are passed have been retained in the gut at body temperature sufficiently long to cause their contained ova to granulate more rapidly after tubing in either dilute hydroxide or water.

[2] Pipettes may be purchased from Dubuar-Rooney, Inc., 207 Water Street, New York City.

zone laterally; then move the preparation with the mechanical stage so that the area just examined is merely out of the field; the adjacent zone is then examined across, and the process continued. If necessary to interrupt the counting it is always done at the end of one of these zones, not part way across.

There are certain advantages in using a shield on the ocular (shown in Pl. XXIII, fig. 1) which cuts off two polar segments (what might be called the arctic and antarctic zones) in the circular microscopical field, and contains a hair running through the longitudinal centre (north to south pole). This is rather more helpful than a shield with a square aperture which cuts off areas laterally in addition. Inasmuch as the preparation is examined in zones traversed laterally there is no advantage, and some disadvantage in restricting any visibility in these portions of the microscopical field. Such shields are easily made out of thin cardboard with a scalpel or razor blade, and a human hair.

As the fields are examined under conditions of high visibility, it is advisable to cut down the diaphragm opening to a minimum.

For *Haemonchus* ova a magnification of 50 diameters (5 × ocular, 16 mm. objective) is serviceable. In mixed infections with small nematode ova a lens combination giving larger magnification may be desirable.

The accurate counting of the ova in a preparation of high visibility (cf. Pl. XXIII, fig. 1) is theoretically the simplest of procedures. Until practice is had to count *all* of them, however, it is actually difficult. It has been a continual surprise to the author during the past several years to observe the degree to which highly visible nematode ova may pass under the eye of an unskilled counter without registering. Attention has previously been called to this by Stoll and Hausheer (1926) in regard to ova of the hookworm of man, and the fact obtains for the larger ova of *Haemonchus* and *Bunostomum* of sheep. Occasional checks against more skilful counters, or the mapping of a microscope preparation by means of the scale on the mechanical stage, and subsequent re-examination of the cover for checking may reveal to just what extent this is occurring. A corollary of this is the high degree of accuracy which is obtained after some practice. The same problem applies, of course, in other counting techniques in which the microscope is used, as for red and white blood cells. In fact, it is our experience that persons accustomed to making blood counts accomplish accurate egg counts with little practice.

A further factor determining accuracy in egg counting is fatigue. Stiles' standard (Boeck and Stiles, 1923) of being "unalterably opposed to more than 3½ hours work per day by a microscopist, except, of course, in a severe emergency" indicates the importance of fatigue in the accuracy of diagnosis for protozoa using the oil immersion. With the low power magnifications used in egg counting and its consequent lessened eye strain, more hours per day can be profitably utilised, but there should be interruptions sufficient to preserve keen vision.

6. Provided the technique is manipulated uniformly and skilfully, determinations of the number of ova per gram of the original faeces may be made with very considerable accuracy (as tested by the best controls now known) by counting an indefinite number of slides from a given flask. In practice, however, it is usually desirable to count the minimum number of slides consistent with reasonable accuracy in the counts. As a working rule, therefore, sufficient slides are counted so that the average count deviates 5 per cent. or less from the average reckoned without benefit of the last count made. Testing will show that once such an average is obtained, its deviation by more than 5 per cent. through the counting of additional drops is rare. Thus, if two drops show respectively 21 and 18 ova (average 19·5) and a third 22, the average of the three drops at 20·3 eggs differs less than 5 per cent. from 19·5, and is an acceptable average. If a fourth drop should show any count between 16 and 24 ova it would not cause the average of all four to deviate by more than 5 per cent. from the already accepted average of 20·3; if, however, it were less than 16 or more than 24 ova, additional slides would be counted. All counts enter the final average, no slide being discarded unless there is an error in the technique adjudged without reference to the count itself.

A summary of our data over a period of months, of preparations showing from 1 to 2691 eggs to the slide, showed from 2 to 8 with an average of 3·4 slides counted from the dilution flasks examined. When the data on 262 flasks were retabulated as to the number of slide counts required to first reach the 5 per cent. deviation noted above, this averaged 2·9 slides. A routine of 3 to 4 slides to the flask is thus our typical counting procedure.

The accepted average slide count multiplied by 200 represents the number of ova per gram of the faeces originally tubed. That is, with 10 grm. of dung in the 300 c.c. of flask contents, each gram is represented by 30 c.c. or 30,000 c.mm. of which 0·15 c.c. or 150 c.mm. represents one two-hundredth part. The hypothetical average slide count of 20·3 represents therefore 4060 eggs per gram, recorded as 4100.

The whole problem of dilution counting of helminth ova in faeces is theoretically very simple, but practically has a certain number of difficulties. This is apparent not only from first-hand observation in the laboratory, especially when workers hitherto unfamiliar with the technique are being instructed in it, but also from the analysis of certain aberrant published results in connection with it. Thorough comminution, a good suspension before promptly withdrawing the drop, its prompt removal and transfer to the slide, and accurate counting on the slide are certain of the points that especially require attention.

Monnig (1928) in South Africa has recently described an adaptation of dilution counting for sheep faeces, in which he observes that "decinormal caustic soda was found to be an excellent diluent and the advantages claimed for it by Stoll are very real." In Monnig's adaptation the sheep pellets are crushed before tubing, the 1 : 15 ratio of faeces-diluent is used, and prepara-

tions are counted without cover slips. In the author's preliminary experiments with sheep dung a variety of modifications of the technique were tested, which touched all of these points and others, but they were later excluded as less satisfactory than the procedures described.

So far as information is now available, all nematode ova which occur in faeces are countable by the dilution method. This appears to be true also of trematode (fluke) ova as well. Cestode "eggs" require a different diluent however. Moreover, the method as described is usable without modification for goat and rabbit dung, and with slight changes may be used on the faeces of perhaps any mammal. Unless it be determined for any type of egg that the diluent actually destroys it, the problem of using a dilution technique is, as Monnig, *l.c.*, states, essentially "to adapt the method to the different types of faeces and not at all to the eggs in them," for the principle of dilution counting is merely the sampling of a dilute faecal mixture.

Flotation counts.

While properly applied a dilution technique, counting a large number of slides, permits the determination of the egg content of sheep faeces with even a small number of ova (*i.e.* 100 or less per gram), it reaches the point of diminishing returns with these low counts by reason of the time required to examine a sufficiently large number of slides to secure a relatively inflexible average. This is a point of rather more importance with herbivorous hosts, which pass large masses of faeces daily. In man, an adult average daily output of formed faeces has been shown to range from 100–150 grm. (Cort, Stoll and Grant, 1929). Adult sheep, on the other hand, expel nearly ten times as much. A female worm living in the adult human gut and producing 10,000 eggs in a day may be expected to show about 100 eggs per gram of formed stool; a worm in an adult sheep's gut, on the other hand, with a similar daily egg output, would show only about 10 eggs per gram of pellets. The problem of the ease or efficiency of determining such low counts merges into the question, not as to how many ova are present in the faeces, but whether any at all are present. No technique known to the author ideally realises this latter requirement under routine conditions. It is probably most nearly approached by Lane's D.C.F. (direct centrifugal flotation) method, checked on suspected samples of appropriate types of infection by culture. It has been the routine of this laboratory to utilise an adapted D.C.F. technique, as hereinafter described, for a control counting method at count ranges between 400 and 1000 eggs per gram of faeces, and to rely on it more particularly on counts less than 400 per gram. The details were settled upon after a preliminary study of over 300 D.C.F. tubes. Certain observations on its comparative accuracy with dilution counts will be noted later in this article. Determinations by D.C.F. are especially serviceable in detecting dawning and disappearing experimental infections, where it is the presence or absence of any ova which is of interest.

The principle underlying D.C.F. is to remove by an initial centrifugation of a faeces-water mixture, a large part of the floating debris and deeply pigmented fluid, and then on centrifugations of the sediment (which holds the eggs) with a solution of high specific gravity, float these eggs and remove them *en masse* on a cover slip for examination with the microscope.

In a recent publication[1] Lane (1928) gives the following description of D.C.F.:

"1. Measure 1 c.c. of faeces. 2. Place it in the special centrifuge tube. 3. Fill the tube with water to within an inch of the top. 4. Perforate the faeces. 5. Cork and shake to obtain complete primary disintegration. 6. Centrifuge adequately. 7. Pour off the supernatant fluid. 8. Nearly fill the tube with a three-quarters-saturated solution of common salt. 9. Obtain secondary disintegration of the centrifugal precipitate. 10. Replace the tube in the 4-horned bucket. 11. Add three-quarters-saturated salt solution till full. 12. Lay a cover glass upon its mouth. 13. Be satisfied that cover and tube are in close contact. 14. Again centrifuge adequately. 15. Pick off the cover fairly sharply. 16. Prepare it as a hanging drop. 17. Herd. 18. Examine."

The details of the procedure as it has been in use on sheep faeces are as follows:

I–III. The first three steps follow identically those for the dilution technique as given on page 117, *except that water is used as the diluent.*

IV. When the flask mixture has been sufficiently shaken to free the ova from adherent debris and distribute them evenly through the water mixture, 15 c.c. are poured promptly into a tube (marked at the 15 c.c. level) of a Lane 4-bucket centrifuge. The other three tubes of the centrifuge are similarly filled. Usual practice is to examine duplicate tubes from each flask.

V. The tubes are now centrifuged for 1 minute at 1000 r.p.m., the centrifuge allowed to decelerate to a full stop "by itself," and the supernate poured off. Under typical conditions there remain about 2 c.c. of debris in the bottom of the tube.

VI. Each tube is nearly filled with saturated sodium chloride solution (sp. gr. 1·200), twirled gently to cause debris to loosen in the bottom, replaced in the tube carrier and completely filled to a level surface by adding additional salt solution with a pipette. This proper filling is made easier by adjusting a light so as to cause a total reflection from the surface. An 18 mm. square cover slip is now gently dropped into place. If properly performed there is no overflow of fluid out of the tube and a small air bubble underlies the cover.

VII. Again the centrifuge is run for 1 minute at 1000 r.p.m. and allowed to decelerate to a full stop "by itself." Each cover is lifted with a little snap, and transferred with its adherent drop of fluid to one end of a slide (thus permitting each slide to mount 2 cover slips).

[1] This article is especially interesting for in it Lane not only reviews in considerable detail his numerous earlier publications, but classifies all other work in the same and cognate fields.

VIII. To the tube is added an amount of salt solution equivalent to that removed on the preceding cover, following which a new cover is placed in position and operation VII repeated. Additional covers, after the same fashion, may be removed until the tube no longer furnishes ova. For a while we used a small clean wire before adding salt solution to stir up the sediment as a method of releasing ova which might have been held there, but this does not appear consistently to increase the percentage of ova secured on the second or succeeding covers, although it may do so. Our usual routine is to examine at least 3 covers.

IX. The slides with their numbered covers (which in the interim have been placed under glass culture dish lids moistened with water on the inside) are now examined *in toto* with the microscope and the aid of a mechanical stage. The ova, floating in the salt solution (Pl. XXIII, fig. 2), underlie the lower surface of the cover. The total number of ova secured from 1 tube represents one-half the egg count per gram of faeces originally placed in the flask.

Except for the evidence of occasional destroyed eggs due to the action of the salt solution (Text-fig. 1) and the tendency of the latter to crystallise at the ordinary humidities of this region, the D.C.F. technique has been surprisingly effective in floating *Haemonchus* ova and those of many other gastro-intestinal nematodes in sheep faeces. Using the routine described, for instance, *Haemonchus* ova are rarely carried into the pour-off at all and preparations are more free from debris than is true when human faeces are examined for hookworm. Certain features of the technique require emphasising, however, if discordant results are not to intervene. These will be best understood when the theory involved in the use of Lane's technique is kept in mind. Ordinarily in centrifugation one seeks the material thrown down in the bottoms of the tubes, or some large fraction of the supernate above such sediment. In the critical salt centrifugations in D.C.F. one seeks rather that very shallow "layer" of fluid and its contained ova which is uppermost in the tube, and his success with the method lies in bringing it off holding as large a percentage of the ova present as possible, and without excess debris. Much of the finely particulate debris which obscures visibility in microscope preparations and the small seeds and objects of similar size which appear in the faeces of animals, especially ruminants, are removed by the first centrifugation. In ordinary centrifuging the matter of balancing tubes is a routine and simple affair, usually performed by adding fluid to the tube or its carrier bucket. In the Lane technique, however, balance must inhere in the tubes and carriers of the centrifuge, for the tubes themselves are manipulated when entirely filled with salt solution.

The cautions here considered, which repay the effort to observe them in the consistent functioning of the method, may be grouped about five points, *i.e.* the centrifuge itself, the centrifuging process, salt solution, covers, and adherent water at the top of the tube.

1. The centrifuge we have used is that especially made for this technique[1]. Care must be used to keep the apparatus well oiled and smooth-running, for when it decelerates slowly and without interference distinctly better flotations are secured. The time required for the slowing down to a stop from 1000 r.p.m. in our centrifuge is a full 2 minutes.

In order to insure proper balance of tubes and carriers, they are all numbered permanently with etched marks, and always used in the same grouping. This is so arranged that carrier 1 and its tube balance to a fraction of a gram with carrier 3 and its tube opposite; likewise carriers 2 and 4 and their tubes are balanced against each other, and with 1 and 3. These balanced conditions obtain not only for the tubes when level full with salt solution and carrying a cover slip, but also for the initial centrifugation with 15 c.c. of faecal mixture in water. As glass centrifuge tubes often vary a good deal in weight, it usually requires a selection from a batch of tubes to secure adequately balanced groupings. The excessive wear and tear on a centrifuge in which the tubes are not balanced is obvious.

The glass tubes should be smoothly ground on top. If a nick occurs in the rim, the tube is useless until reground (as on a carborundum stone), a process which deserves some care lest the cover slip fails to fit exactly around the rim. After tubes are reground they require rebalancing.

2. A point of value in maintaining high efficiency with D.C.F. is to have an even swing of the centrifuge handle throughout the minute it is run at 1000 r.p.m. Technicians have usually found this a matter requiring considerable practice before skill is obtained. Any jerkiness in the movement of the centrifuge head, whether communicated to it by the operator's unskilful manipulation during spinning or when disengaging the handle, or by inadvertent touching of the head or tubes while still in motion, produces poorer flotation efficiency.

3. While saturated sodium chloride solution has certain disadvantages through its tendency to crystallise rapidly at low humidities, its action, at least occasionally, in destroying eggs through its strong osmotic force, and its corrosive action on the centrifuge, it appears nevertheless to be a serviceable flotation fluid. Provided examination of the faecal mixture is not performed too soon after tubing and crushing of pellets, difficulties with the mass of fine bubbles which sometimes form a complication with the technique may be almost entirely avoided by using a truly saturated salt solution (easily prepared by boiling the salt solution until a crystal scum forms, cooling, filtering, and using cool) which is never poured rapidly into a tube or squirted from a pipette. A three-quarters-saturated solution works nearly as well for certain purposes but yields a somewhat lower per cent. of the *Haemonchus* ova on the first covers, requires the examination of more covers to secure all the ova procurable from a given tube, and is less useful in floating smaller nematode ova. Nor are the disadvantages mentioned with saturated salt solution absent.

[1] By R. B. Turner and Company, London, England.

4. Lane has mentioned, but, in the opinion of the writer, insufficiently emphasised, the importance of using very well-cleaned cover slips. Instead of the thick covers which are a part of the standard apparatus, ordinary 18 mm. square covers, No. 3 (or No. 2) thickness are employed. These are cleaned in acid cleaning solution, well rinsed in water, then carried through 95 per cent. to absolute ethyl alcohol where they remain until dried. This is done preferably by flaming, rather than with a cloth and only as needed for covering the tubes. Such covers should wet easily with salt solution and are imperfectly cleaned unless they do, and should be handled with tweezers until ready to drop on the tubes.

In removing covers from the tubes after centrifuging, rather than lifting them by diagonal corners, they are grasped at two adjacent corners by the thumb and forefinger, lifted rather sharply in order to secure as large a drop as possible from the top of the tube, and then gingerly transferred to the $1\frac{1}{2} \times 3$ slides which have been numbered with wax pencil according to the carriers. Properly filled tubes, as earlier mentioned, show a small bubble under the cover before centrifuging. If the centrifugation process has been well done this bubble should show no increase in size before the cover is lifted; increase in size of bubble indicates loss of fluid during centrifuging and thus potential loss of ova. Moreover, once the centrifuge has come to rest it is of importance not to move the tubes in such a way that the bubble moves around under the cover slip. In appropriately heavily loaded preparations, the path of the bubble may be traced by the ova it has pushed away from the nether surface of the cover and into the underlying fluid.

5. In our earlier experience there occasionally appeared preparations in which the first cover delivered only a small number of eggs, but the second cover a large number. The solution of this difficulty was obscure for a very long time, until it was discovered that occasionally in pouring-off the supernatant water following the initial centrifugation a small drop adhered near the top of the tube. Later, when the tube was filled with salt solution and the cover slip added, this water would wet the underside of the cover and dilute enough of the upper layer of fluid to destroy its possibility of retaining a large percentage of the ova on the first cover. Once noted this difficulty was easily and routinely resolved by wiping the sides of the tube near the top after the pour-off and before the salt solution was added. Incidentally it may be noted that with sheep dung preparations, the tube at the pour-off may be entirely inverted momentarily to drain out practically all free fluid, without disturbing the ova which are held in the debris in the bottom of the tube.

Details have been purposely emphasised in the discussion of D.C.F. as adapted to sheep faeces for *Haemonchus* ova, for it is the author's conclusion that such consistently reliable results as the technique is able to furnish here cannot be obtained without care in regard to them. An experience covering thousands of preparations with the technique, either personally or under his eye, assures him that while vagaries in its functioning occasionally occur it

can be made to function more precisely than any other egg-*finding* method for routine laboratory work, and serves excellently for the smaller egg counts.

Our experience is affirmative, therefore, in regard to the value of a flotation method on sheep faeces, agreeing in that respect with Smith and Ring (1927) rather than negative as Monnig (1928) has reported. Moreover, when handled as described the method appears to be a serviceable diagnostic procedure for examining many kinds of faeces[1] for the ova of the common strongyloid nematodes and many cestodes which are present in the dung of sheep, goat, cat, dog, rat, rabbit and pig. Coccidia and various protozoan cysts appear to be likewise effectively floated. Generally speaking, it shows poor results for *Ascaris lumbricoides*, *Trichuris* spp. and allied forms, and is probably valueless for trematode ova. Because D.C.F. relies on the capacity of a given kind of egg to float in a fluid of given specific gravity, it is a technique which must be adapted not only to a given type of faeces, but to the particular kind of eggs which it is desired to float from them.

ANALYSIS OF D.C.F. COUNTS.

With the use we have made of both D.C.F. and dilution counting as quantitative methods in enumerating *Haemonchus* ova in sheep dung, it is profitable to survey the functioning of the two techniques under routine conditions.

It will be noted in connection with the description of D.C.F. that our use of the method produces about 2 c.c. or less of debris in the tube, which, with the diameter of the tubes used, gives a depth of about 1·2 cm. sediment. As these tubes in general measure about 12 cm. long, the ova-containing debris represents thus about 10 per cent. of the total depth of the tube. These conditions obtain for the following data. When this ratio is much exceeded ova are not well floated, in our experience.

Pour-offs. It is one of the fortunate conditions in the use of D.C.F. for *Haemonchus* ova in sheep dung, that eggs are not carried appreciably into the pour-off. In examining the pour-offs of 141 tubes, 36 were from tubes that showed no ova, and the pour-offs were also negative. Of the other 105 tubes 104 had negative pour-offs. The one positive pour-off contained 6 ova on one cover, the main tube containing 251 ova. (As this happened with a new technician the possibility of a contamination is not excluded.) From these 105 tubes there were floated a total of 28,565 ova (range 1 to 1947 per tube), or a loss in the pour-off of 0·02 per cent. This is a rate too low to take into serious account.

The reason for the failure of *Haemonchus* ova to go into the pour-offs probably lies in the fact that they tend to go down upon centrifugation somewhat more rapidly than the light herbivorous faecal debris, and thus are held in the lowermost part of the tube. This has permitted, as a routine matter in fact, the complete upending of the tube in pouring off, mentioned earlier.

[1] "Milk diet" faeces give poor flotation results.

This manipulation, having been found possible, obtained with the majority of the above tubes.

"*Negative*" *tubes.* All negatives over a period of several months were summarised. Of 460 tubes, negative on the first cover, 458 were also negative on the second; the 2 positive second covers showed respectively 1 and 4 ova. Similarly of 39 tubes negative on the first cover, 38 were likewise negative on the second and third covers, and only 1 tube showed an egg on the second cover, followed by a negative on the third. Thus of 502 tubes negative on first cover, 499 or 99·4 per cent. were negative on succeeding covers. While failure to find ova is literally "negative" evidence, taken in connection with the high percentage of *Haemonchus* ova usually secured on the first and second covers (*vide infra*), it raises the presumption very high indeed that there was absence of ova in by far the greatest percentage of these tubes. An additional check on this probability lay in the fact that, in the majority of cases, more than 1 tube was made from a given flask.

Two other kinds of evidence are available on this point—the continuance of negative after first getting positive covers; and the results of cultures. Concerning the first, if tubes containing ova are examined on successive covers until negative, only rarely do straggling ova appear on additional covers. Thus 4 tubes from the same flask examined together, showed the following results on 8 covers each: Tube *A*, 99 *Haemonchus* eggs first cover, 8 second, then 6 negatives; Tube *B*, 100 first cover, 2 second, 1 third, none fourth, 1 fifth, then 3 negatives; Tube *C*, 107 first cover, 1 second, then 6 negatives; Tube *D*, 87 first cover, 8 second, then 6 negatives. In this case, Tubes *A*, *C* and *D* after showing 310 ova on the first two covers per tube were negative on six additional, and only 1 egg appeared in Tube *B* after it first became negative on the fourth cover.

Culture tests show somewhat similar results. From 13 different faecal collections (representing 10 sheep) 64 covers were examined from 32 D.C.F. tubes, and all found negative. From the identical faecal samples cultures were made, after the manner of White (1927). These cultures utilised from 5 to 100 grm. of dung each, a total of 650 grm. being cultured. After appropriate periods (usually 1 to 2 weeks) the culture masses were isolated in the Baermann apparatus and the dishes washed out with water, the washings being sedimented. No *Haemonchus* larvae could be demonstrated (two of the cultures contained a total of 5 free-living nemas, evidently from the tap water). Five other cultures of 100 grm. each, representing 4 sheep, showed respectively 2, 3, 9, 10 and 46 larvae. The specimens from which the cultures were made had been originally examined with 28 D.C.F. tubes, 2 covers to a tube. In fact, for one of these specimens (that showing 3 larvae in 100 grm. on culture) the entire 300 c.c. flask and washings from it had been examined with 22 D.C.F. tubes, 2 covers each, and 18 pour-offs, all being negative. These positive cultures indicate that a D.C.F. negative may occasionally mask an actual positive, although our data likewise indicate that this cannot be con-

sidered too serious a reflection upon the method. For instance, other culture studies show that about 10 per cent. of the ova put into a culture are recovered as larvae: this would mean respectively about 20, 30, 90, 100 and 460 ova in the cultures above, or 0·2, 0·3, 0·9, 1 and 4·6 ova per gram. In a D.C.F. flask made up with 10 grm. of faeces there would thus be 2 to 46 ova in the flasks, provided the ova are distributed as uniformly through the dung as by other studies we are led to believe they are. If the tubes poured from the flask for D.C.F. are fair samples of the flask contents, there should have been about 2 ova per tube in the highest noted above. In four of the above cases it would easily have been possible, by the law of chance, to have poured one or two of the twenty 15 c.c. D.C.F. tubes in a 300 c.c. flask containing totals of 2 to 10 ova and secured not an egg; or with eggs so low in number as to average but 2 to 10 in 10 grm., it is conceivable that the entire 10 grm. taken from a large faecal sample for the D.C.F. flask would occasionally fail to contain ova. Such an explanation would be much less likely to hold for the faeces calculated to contain 46 ova in a 10 grm. sample, however. In all of these cases likewise the explanation is permissible that occasional loss of ova obtains regularly through their destruction by saturated salt solution. In conclusion then, while of 18 faecal specimens negative to D.C.F. 5 were positive to culture, only 1 of these contained enough eggs (theoretically 2 in a D.C.F. tube) to have surely permitted their demonstration.

Percentage of ova on first and second D.C.F. covers. The relatively high efficiency of the D.C.F. technique in accounting for *Haemonchus* ova in sheep dung is revealed by the percentage of all the ova which appear on the first two covers from a tube. In the analyses which follow, the tubes represented are typical series, usually being all of a given category from our records over a certain period of months. (Not all the tubes, as will be noted, are run in our routine to complete negatives, the final additional number of eggs being approximated on the basis of the findings to be given.)

When the total number of ova secured from a D.C.F. tube is from 1 to 9 eggs, it is the rule for them all to appear on the first two covers. Thus, of 153 such tubes, in 123 all the ova were on the first cover, the second being negative (and in 60 of them where precautionary third covers were examined these were likewise negative). In another 20 tubes, all the ova were on the first two covers, the third being negative. The remaining 10 tubes, positive on the first and second, were not run to a third cover.

When 10 or more ova are secured from a tube, there is slightly more lagging in their appearance, depending partly on their total number, but a surprisingly high percentage nevertheless appear on the first two covers. In a series of 1004 such tubes, 274 were run to negatives. Of these 34 were negative on second cover (of 19 run to a precautionary third cover, all were likewise negative), 214 on third (of 20 run to a precautionary fourth cover, all were likewise negative) and 26 on fourth. Those with all the ova on the first cover ranged from 10 to 571 (average 48) per tube; those with all ova on the first

and second covers ranged from 10 to 649 (average 124) per tube; and those becoming negative on the fourth cover, with 98·1 per cent. on the first two covers, ranged from 92 to 652 (average 223) per tube. Certain additional tubes had so few ova on third, fourth or fifth covers as to represent 1 per cent. or less of the total from the tubes. As our general experience indicates that once a cover yields so few ova, no greater percentage would appear on additional covers, it is possible to utilise totals from such tubes as representing to a fraction of a per cent. all that would be secured. Of 88 such tubes, with 1 per cent. or less on the third cover, the first two covers yielded 99·3 per cent. of the total, and of 59 tubes with 1 per cent. or less on a fourth or succeeding cover, the first two covers yielded 96·8 per cent. of the total. The former group showed 60 to 746 ova (average 304) per tube, the latter 79 to 2671 ova (average 420) per tube.

The high percentage of ova from the first two covers is reflected in the yields on the first and second covers considered separately. The 214 tubes negative on third cover showed 90·1 per cent. on the first cover, 9·9 per cent. on second. Lumping the three groups of tubes which had 1 per cent. or less on the third or fourth covers, or were run to a negative with 4 or more covers, shows that these 173 tubes delivered 87·3 per cent. on the first and 11·0 per cent. on the second covers. As these are skew in the direction of over 90 and less than 10 per cent., probable errors would not enhance their value particularly. It may be pointed out, however, that the occasional tubes with low yield on the first cover quite uniformly compensate on the second cover. An example will illustrate this. Of 2 tubes poured from the same flask, 3 successive covers from each tube yielded respectively 94, 21 and 1 on the first tube, 5, 113, and 4 on the second tube, totals of 116 and 122 ova.

With such a large percentage of *Haemonchus* eggs appearing on the first two covers, it is possible on the basis of the above results to utilise counts from tubes which are not run to a negative as complete totals from such tubes by merely adding a percentage of the partial total secured on the first two covers, provided the yields on these first two covers are in approximately 9 to 1 ratio. Thus in the group of 173 tubes above, 98·3 per cent. of the ova appeared on the first two covers. Adding 2 per cent. therefore to the total secured on 2 covers produces very closely the total which would have been secured by examining additional covers. The small error which may be involved in the occasional tube total approximated by such means would not influence an egg count per gram interpretation seriously, for significant differences in egg counts are of a different order of magnitude. As already noted, however, our usual routine is to examine 3 covers.

Comparative yields of ova from tubes poured from same flask. With the D.C.F. demonstrably producing high yields of *Haemonchus* ova on the first few covers examined from a tube, it remains to show whether successive tubes from a given flask produce similar total counts. One way to illustrate the type of results secured is by specific examples. Thus 3 tubes from the same

flask yielded in the order of their pouring respectively 245, 239 and 258 ova, and another set of 4 from a flask from another animal 452, 417, 448 and 429. These are clearly comparable to a high degree. More explicit statements may be made on longer series, either by comparing the yields from the 20 tubes which a single 300 c.c. flask will fill, or a longer series of flasks from which 2 tubes only were examined.

As illustrations of the former, from 2 flasks, *A* and *B*, made up from 2 sheep, all the ova were counted. Besides the 20 tubes which utilised to 300 c.c. of faecal mixture, the interiors of the flasks were washed and the washings likewise examined until ova could no longer be demonstrated. The total count from flask *A* was 2185, from flask *B*, 1961 ova, of which the washings represented 44 and 10 ova, respectively, or 2·0 and 0·5 per cent. of all eggs obtained from the flasks. When the two totals were divided by 20 and the actual ova floated per tube expressed as percentages of these 1/20th fractions, it was shown that the mean yield per tube was respectively $97{\cdot}9 \pm 1{\cdot}5$ and $99{\cdot}5 \pm 1{\cdot}7$ per cent. of the expected numbers. The coefficients of variation which are of more interest were $10{\cdot}1 \pm 1{\cdot}1$ per cent. for *A*, $11{\cdot}1 \pm 1{\cdot}2$ per cent. for *B*. In other words, the typical variation in yield per tube was within about 10 per cent. of the theoretical demand.

In a series of 130 flasks from which 2 tubes each were examined, the total ova count from each second tube was expressed as a percentage of those from the first, and these ratios treated as a frequency distribution. The ova secured from the second tubes represented $100{\cdot}2 \pm 0{\cdot}8$ per cent. of those secured from the first, with a coefficient of variation of $13{\cdot}7 \pm 0{\cdot}6$ per cent. These tube totals ranged from 21 to 728 ova, the average number being 172 ova.

This similarity of yield between tubes from the same flask, establishes then, the basis of the D.C.F. as a counting technique for *Haemonchus* ova.

Comparison of D.C.F. and dilution counts. The foregoing analysis represents our routine expectation of D.C.F. as a quantitative procedure for *Haemonchus* ova in sheep dung. It has not answered the question as to whether the highly consistent counts demonstrable actually represent the entire number of ova in the faeces examined. The direct proof of such a question is difficult to secure, as some of the literature on D.C.F. and dilution counting of hookworm ova in human faeces attests. Three considerations, for instance, give point to the problem.

In the first place, water does not appear to be a completely satisfactory diluent in which to comminute sheep faeces. Not only does the dung fail to break up into particles as fine as those in a dilute hydroxide suspension (as is easily demonstrated by the difficulty of drawing a pipette sample from a water flask as compared to a dilution counting flask made up with decinormal sodium hydroxide), but bacterial action proceeds at an accelerated rate in water suspensions of faeces. Both of these factors would theoretically permit the withholding of ova from D.C.F. covers.

Again, there is the direct evidence as in Text-fig. 1 that D.C.F. at least occasionally destroys eggs. As this can be demonstrated at unpredictable

times in routine D.C.F. cover-slip preparations examined under the microscope, it remains a question as to whether it may not be true of ova which never reach the surface film, but "pop" beforehand and then sink to the debris which does not float. In Text-fig. 1 it will be noted that in the case of each burst egg, the hole in the shell through which the protoplasm is pushed appears to be plugged by it, thus permitting the partially emptied egg to still retain sufficient buoyancy to float. If the protoplasm had been forced entirely out of the egg shell it is very probable that both would have been *spurlos versenkt*. It is thus possible that certain ova in most or all D.C.F. tubes may be lost.

Text-fig. 1. Hanging drop D.C.F. preparation mounted on plasticine mounds, showing in one field 5 ova which have burst through strong osmotic action of flotation fluid. The extruded protoplasmic contents in each case plug the opening in the egg, permitting them still to float. Magnification ×130. D.C.F. preparation 5 days after tubing with water.

While our preparations are habitually examined with the cover slip flat on the slide, when burst ova are found they may be readily demonstrated in hanging drop preparations as well from the same flask contents, which the photograph illustrates. Cover-slip pressure is thus ruled out as the cause. These burst ova, moreover, are likewise encountered when salt solution of 1·150 rather than the recommended 1·200 is used. The reason why eggs from some faecal mixtures show the condition more than others is not yet clear, although the phenomenon is more frequently demonstrable in flask material which has been left standing at room temperature a week or more.

The third consideration is that a total count of ova from a preparation, provided it fails to demonstrate the true number of eggs present, must always produce a smaller than the correct number. There are no balancing factors as in dilution counting, where if one pipette drop from a flask should by chance secure a smaller than a proper proportionate number of ova, another drop should by chance as likely secure a larger than the proper proportionate number, etc., so that the final accepted average has been compensated. With any total counting method, as D.C.F., this compensation does not enter, except to the extent that additional tubes are counted from a given flask, and regular dilution principles become applicable.

As a check on the actual accuracy of the D.C.F. counts as we employ it, there have been utilised regular dilution counts from flasks prepared from the same faecal sample. It may be objected that two 10-grm. lots of faeces from a given collection from a sheep, one made up for dilution count, the other for D.C.F. tubes, may not contain exactly the same number of ova. So far, we have been unable to prove th[illegible]is f[illegible]tor exists. Variations have, in other words, not been greater than the usual counting error by either method. Or, that the 15 c.c. D.C.F. tube poured from the 300 c.c. flask may happen to be a low variant. In counts compared from a series of faecal samples, any such effects would be balanced. A more tang[illegible]le difficulty exists in the fact that the most effective ranges of the two techniques overlap only slightly, which is the reason, of course, that the two are employed in experimental studies. High egg counts naturally favour the dilution method, low counts the D.C.F.

Over a period of several months, a series of comparative counts were secured on 43 faecal samples showing more than 200 ova by D.C.F. (*i.e.* 400 eggs per gram, and thus theoretically 2 or more ova to a dilution slide). The D.C.F. total counts ranged from 205 to 728 per tube, averaging 355, the number of ova counted from these tubes being 15,255. The comparative dilution averages from these same tubes demonstrated $111{\cdot}6 \pm 3{\cdot}2$ per cent. of the D.C.F. counts figured as ratios of the eggs per gram by each method. Or, reciprocally, on the egg count per gram basis, the D.C.F. demonstrated only 89·6 per cent. of the dilution counts. (Of this group of 43 comparisons, 38 are clustered close to the mean value. If the 5 which are most variable are omitted, the dilution averages are still $106{\cdot}8 \pm 1{\cdot}1$ per cent. of the D.C.F. counts on a per gram basis; this differs from $111{\cdot}6 \pm 3{\cdot}2$ by only $4{\cdot}8 \pm 3{\cdot}3$ per cent., a difference of insufficient significance to warrant discarding the 5 as aberrant results.)

While the series available here is short, nevertheless the evidence indicates clearly that D.C.F. on the average accounts for only about 90 per cent. of the *Haemonchus* ova demonstrable by dilution counts from the same sheep faeces as we employ it.

It is of interest to note that the comparative quantitative result of D.C.F. with hookworm ova from human faeces is slightly less favourable than for *Haemonchus* ova from sheep. Thus hookworm eggs are usually carried into

the pour-offs, Lane's own data (1928), Table XVI, showing an average of 1·7 per cent. per tube, in one case reaching 18·4 per cent., with Stoll and Hausheer, *l.c.*, having had somewhat better results averaging 1·6 per cent., the highest tube having 9·7 per cent. in the pour-off. Again, of the ova recovered on the 1st and 2nd covers, compared to all secured from a given tube (omitting those in the pour-offs), Lane's data, *l.c.*, show 77·8 and 19·1, and the latter authors' 60·0 and 23·0 per cent. respectively. Finally, in the only check approaching adequacy that has as yet been made of comparative hookworm counts by the D.C.F. and dilution methods, Stoll and Hausheer, *l.c.*, proved that the former method demonstrates but 81·4 $\pm$ 1·5 per cent. of the ova per gram as shown by dilution count. The results of D.C.F. with *Haemonchus* ova thus classify as distinctly better than those with *Necator*, which is probably related to the fact that the stomach worm ova being larger, float more perfectly in a fluid of high specific gravity.

It is emphasised that this study has been concerned primarily with *Haemonchus contortus* ova in sheep dung. It is probable that the conclusions may be extended without significant changes to goat dung. It is also probable that in both techniques described, larger ova, such as those of *Bunostomum* and *Nematodirus* will be demonstrable with even greater facility, and smaller ova, as of the smaller strongyles, with less facility. So far as our observations go, the ova of such worms as the various species and close relatives of *Ascaris* and *Trichuris* do not come off well in D.C.F. preparations, although they are readily counted by dilution.

Discussion.

With domestic animals, such as sheep, in which large amounts of dung are passed in relation to body weight, it is clear that the possibility of determining with a high probability of accuracy whether parasite eggs are in the faeces, and if present their number, requires techniques of a standard of excellence comparable perhaps to the culture methods of the bacteriologist. Furthermore, even after the necessary details of such procedures are determined, and assessed as to their value, constant vigilance is required to keep performance at a level of reliability consistent with experimental procedures. The author deprecates the idea that because the helminthologist studies worms, the life-histories of many of which are intimately concerned with the gastro-intestinal tract and its excreta, therefore, less precise care should be expended on the techniques devoted to their study than is true for smaller micro-organisms cultivable *in vitro*. Indeed, quite the opposite is true. The lesson of recent years in the study of certain of the helminths of man is clearly that technical procedures of very considerable value are now available for the worker who is interested in knowing for a given animal something more than merely whether or not it is passing a few worm eggs.

The use of any egg-counting technique as an indirect measure of the size of nematode infestations of the gastro-intestinal tract of the host being studied, rests upon certain assumptions which it may be well to recapitulate briefly.

While it is obvious that these assumptions will need to be verified for each type of parasite and host that is being investigated, enough data have been secured by now to indicate the probability that in the absence of opposite indications it may be accepted as a working hypothesis, under ordinary conditions, that:

1. There is a relatively steady egg flow per day from a stabilised group of worms in a host except in so far as it is upset by the variations in the amount of faecal output (Stoll, 1924).

2. One method of smoothing this variability is to sample two or more consecutive days' total faeces and compute the actual average egg output (Stoll, 1924).

3. Such an average daily egg output affords a measure of the number of worms present in the host at the time (Stoll, 1923 *b*).

4. In hosts passing equivalent amounts of faeces comparisons may be made of the severity of the infestations by comparing their egg counts per gram of faeces (Cort, 1925).

Utilisation of this information has permitted studies of considerable importance on the epidemiology of certain human parasites (Cort, 1925), the effect of curative measures (Faust and Khaw, 1927; Schapiro and Stoll, 1927), the normal life-history of parasitic infestations (Herrick, 1928), etc.

While such studies have been made for the most part on parasites of interest primarily in human medicine, there seems to be no good reason why similar investigations may not be profitable with domestic animals. Moreover, the use of methods which permit observations on the status of any helminthic infestation of the mammalian gastro-intestinal tract and its diverticula, without any disturbance or dislocation of the infestation itself, suggests many possibilities of experimentation that might otherwise be incapable of study, or which might need to be performed in the absence of information which is now readily obtainable, albeit with the necessity of certain equipment and a period of training.

Summary.

There have been described in detail adaptations of dilution egg counting and direct centrifugal flotation for use on sheep faeces for the ova of *Haemonchus contortus*, together with certain data on their comparative efficiency. It is suggested that extension of the methods as given, or with appropriate modifications, would permit studies from a variety of angles on the helminthic infestations of the gastro-intestinal tract and its diverticula among domestic animals.

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EXPLANATION OF PLATE XXIII.

Fig. 1. One field of dilution slide from sheep-dung preparation showing about 18,000 eggs per gram. Note high visibility of ova, and the ocular shield with hair line. Original magnification × 50 (5 × ocular, 16 mm. objective), which is the usual lens combination used for counting *Haemonchus* ova.

Fig. 2. Demonstration of D.C.F. preparation, original magnification × 50 (5 × ocular, 16 mm. objective) illustrating high visibility and typical lack of debris. The ova are mostly *Haemonchus contortus*.

(*MS. received for publication* 18. VII. 1929.—Ed.)

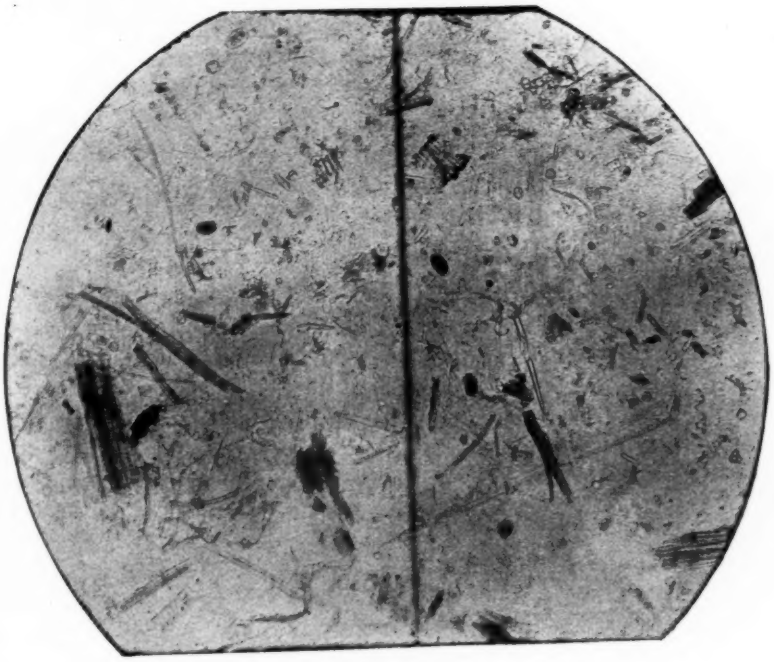

Fig. 1.

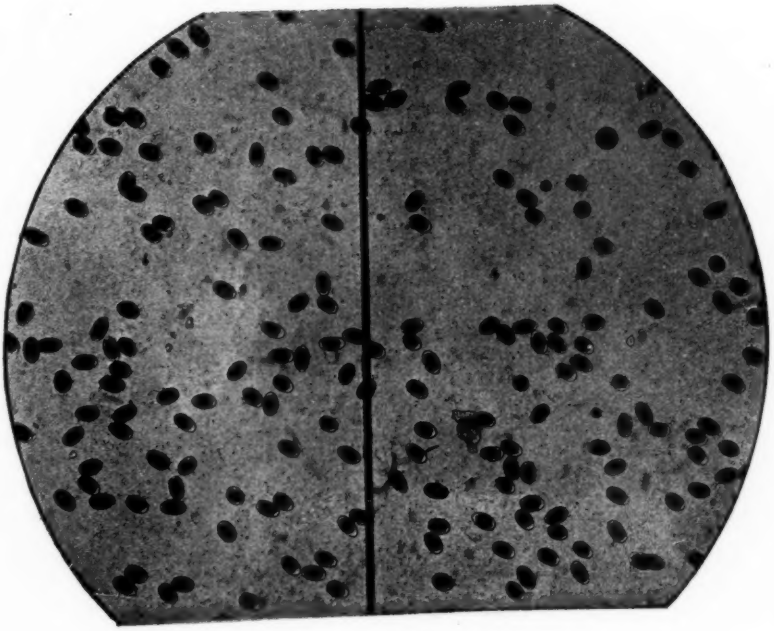

Fig. 2.

UNIV. OF MICH.

FURTHER REMARKS ON THE GENUS *LERNAEOPODA*, INCLUDING A DESCRIPTION OF A NEW SPECIES, *L. BIVIA*.

By W. H. LEIGH-SHARPE, M.Sc. (Lond.).

(With 5 Text-figures.)

I. **L. scyllicola** Leigh-Sharpe, 1915.

Part IV. The male[1].

In February 1928 I obtained from a ♂ *Scyllium canicula*, taken at Plymouth, a ♀ *Lernaeopoda scyllicola* bearing a younger and better preserved male than any I had hitherto observed. It is the first time in this genus that a male has been found anywhere other than on the female's shoulder, namely on one of the abdominal appendages, and the male was probably alive at the moment of fixation. The heads of the sexes point in opposite directions so that in Fig. 1 the abdominal appendage is "upside down," and the genital process of the male is near a vulva of the female which is however fertilised since it bears egg-strings. I am now fortunate in having the kind services of Miss E. C. Humphreys to illustrate this male which is not shrunk into its carapace and desire that Figs 1. and 2 shall supersede my figures of 1918. The near second maxilla has been omitted to show the method of attachment of the male.

The *antennule* (Fig. 2 *A*) is four-articled and the basal joint is more like that of the female than I previously implied. There appears to be a lateral sub-apical projection in addition to the three terminal setae. The penultimate article is the smallest. The *antenna* (Fig. 2 *B*) is figured in a different aspect.

The occurrence of *L. scyllicola* ♀ on male dogfish at Plymouth, represented by dividing the number of male dogfish examined (100–300 per annum) into the number of parasites taken, is as follows:

1914, 4; 1915, 4 (max. 6); 1916, 3; 1917, 1; 1918, 2; 1919, 1; 1920, 0·5; 1921, 0·1; 1922, 0·5; 1923, 1; 1924, 0·5; 1925, 1; 1926, 0·5; 1927, 1; 1928, 1.

II. **L. globosa** Leigh-Sharpe, 1918.

Part II[2].

With more abundant material, and the kind services of Miss E. C. Humphreys and C. L. Oakley, I am now in a position to give improved figures of, and to make a few corrections concerning, the appendages of this species (Fig. 3).

[1] The previous parts appeared as follows: Part I. ♀. *Parasitology*, **8**, 262, 1915. Part II. ♂. *Parasitology*, **11**, 18, 1918. Part III. ♀. *Parasitology*, **11**, 263, 1919.

[2] Part I appeared in *Parasitology*, **11**, 29, 1918.

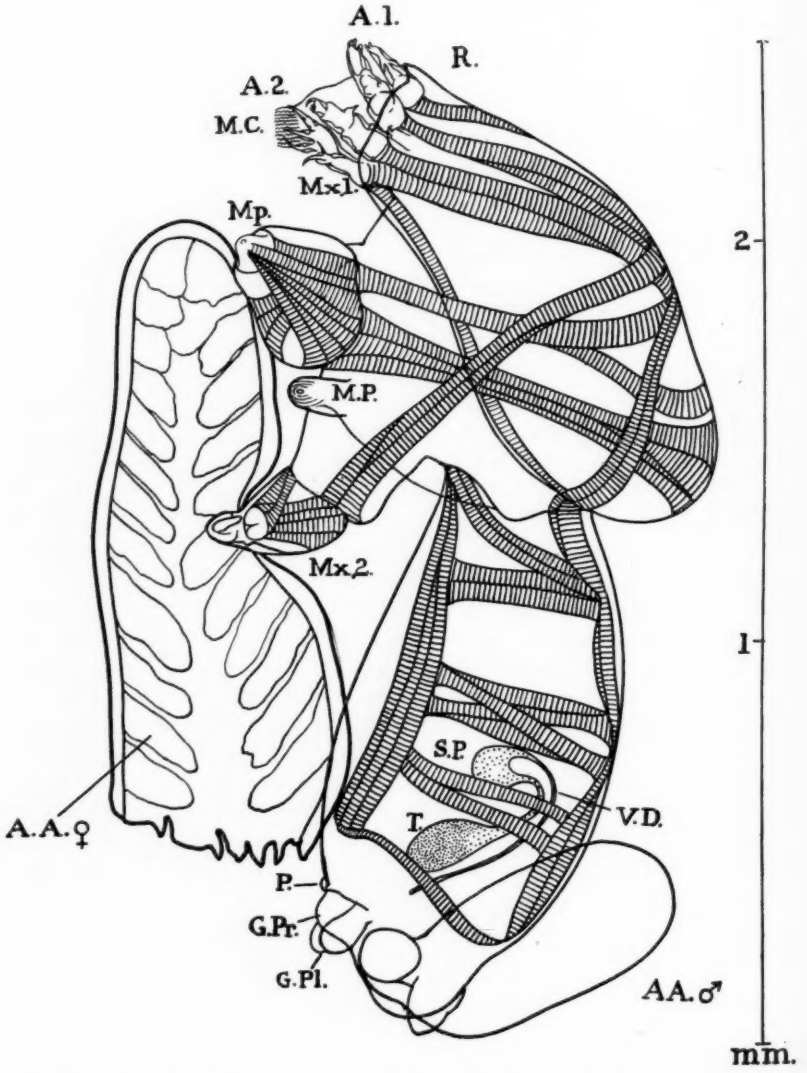

Fig. 1. *Lernaeopoda scyllicola* ♂. *A.A.* ♀=abdominal appendage of female; *A.A.* ♂=abdominal appendage of male; *R.*=rostrum; *A.1.*=antennule; *A.2.*=antenna; *M.C.*=mouth cone; *Mx.1.*=first maxilla; *Mp.*=maxillipede; *Mx.2.*=second maxilla; *M.P.*=mediative process; *T.*=testis; *S.P.*=spermatophorogonium; *V.D.*=vas deferens; *G.Pr.*=genital process *G.Pl.*=genital plates; *P.*=protuberance.

L. globosa is fairly common in the nasal fossae of *Scyllium canicula* at Plymouth and other parts of the English coast, on either sex. It occurs not more frequently than upon one dogfish in ten and not more rarely than upon

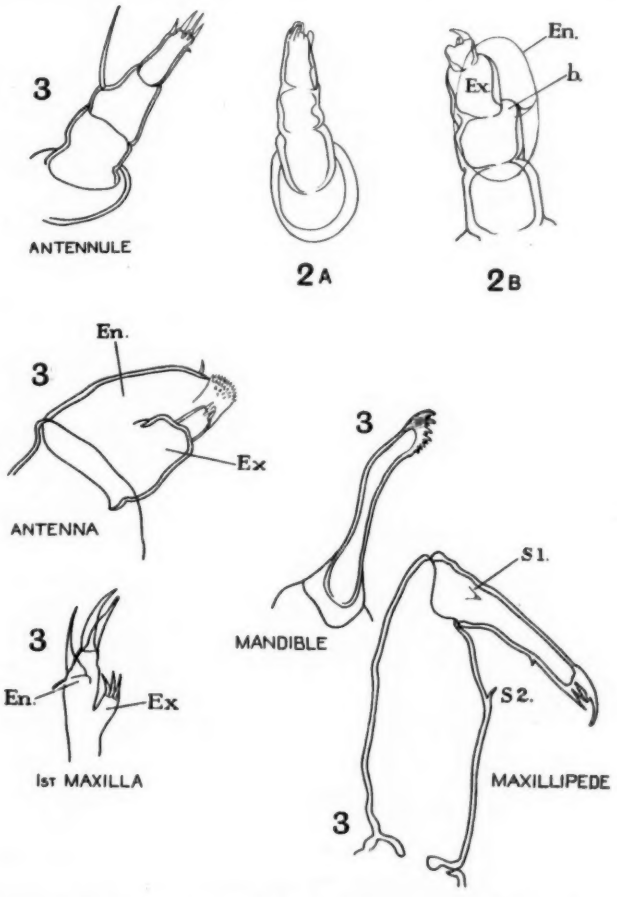

Fig. 2. *Lernaeopoda scyllicola* ♂. *A.* = antennule; *B.* = antenna; *En.* = endopodite; *Ex.* = exopodite; *b.* = basal article of exopodite.

Fig. 3. *Lernaeopoda globosa* ♀, a revised figure of the appendages. *Ex.* = exopodite; *En.* = endopodite; *S.1.* = specific process; *S.2.* = specific spine.

one in twenty. When it does appear several females are as a rule found in one fossa.

It is curious that in the eleven years that this parasite has now been under continuous observation by my assistants and myself not a single male has as yet been found.

The *antennule* is four-articled and terminates in five setae, only the three large ones of which were previously recorded by me. A constant feature which I have previously overlooked, is the presence of an enormously elongated seta on a basal projection of the penultimate article, and which is as long as the rest of the appendage.

The endopodite of antenna ends in a pad of spines.

The *mandible* is curved and has a dental formula of *H* 1, *Z* 1, *H* 1, *Z* 1, *H* 1, *Z* 1, *H* 1, *N* 4. The first and second *maxillae* are as previously described.

The *maxillipede* with its specific spine (Fig. 3, *S*.2) feebly developed is as previously described, but I have now found on the terminal article a small

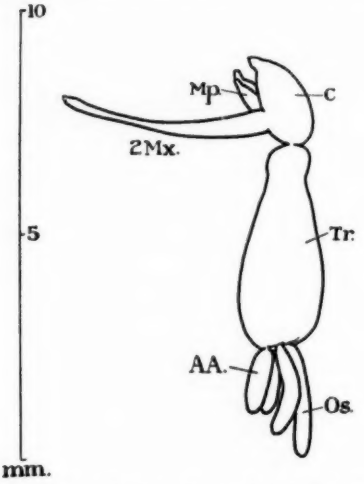

Fig. 4. *Lernaeopoda bivia* ♀, in left lateral aspect. *C.* = cephalothorax; *Tr.* = trunk; *Mp.* = maxillipedes; *2Mx.* = second maxilla; *A.A.* = abdominal appendages; *Os.* = ovisacs.

process bearing a spine and suggesting an exopodite, a condition exactly recalling that of *Ommatokoita superba* Leigh-Sharpe, *Parasitology*, **18**, 224. As such an exopodite has not yet been discovered in any other species of *Lernaeopoda* it may be that, when a male is forthcoming, this species will have to be transferred to the genus *Ommatokoita*.

III. **L. bivia** n.sp.

Material. A single female specimen in the extra-cloacal region formed by the union of the pelvic fins of *Scyllium bivium* ♂ from Orange Bay, Brit. Mus. Coll. (*vide Journal of Morphology*, **42**, 328).

Body. The general outline of the body drawn to scale is best seen in Fig. 4. No other species of this genus has the cephalothorax connected to the trunk by so narrow a constriction, nor the anterior portion of the trunk dilated. The abdominal appendages are large and broad and the ovisacs are but twice their length.

Cephalothorax. Almost in a straight line with the trunk.

Trunk. This is the only species, besides *L. globosa*, in which the trunk is broadest in the middle region, and not posteriorly (cf. *Parasitology*, **11**, 257, Fig. 1). A distinct enlargement anteriorly. Clavate and elongated, not globose as in *L. globosa*.

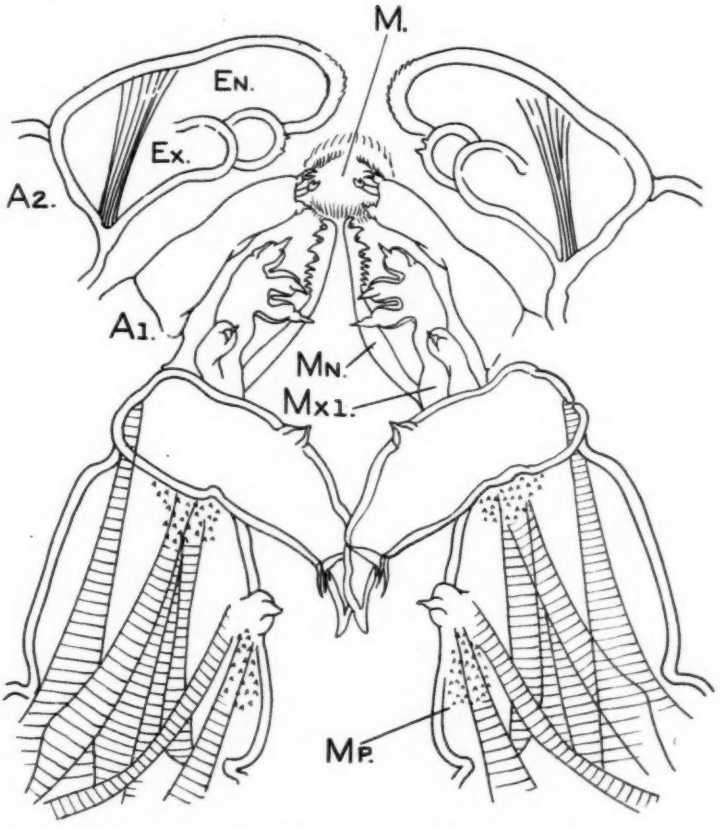

Fig. 5. *Lernaeopoda bivia* ♀. The mouth parts. *M.* = mouth; *A.*1. = antennules; *A.*2. = antennae; *Mn.* = mandible; *Mx.*1. = first maxilla; *Mp.* = maxillipede.

Appendages, all paired, are (Fig. 5):

Antennules. The usual three articles cannot be made out. They are tripartite at the extremity, one ramus being a mammillated spine and the other two bifurcating.

Antennae. Both exopodite and endopodite much rounded, the terminal article of the former being spherical.

Mandibles. Dental formula *H* 1, *Z* 1, *H* 1, *Z* 1, *H* 1, *Z* 1, *H* 1, *N* 4.

First maxillae. As in other species.

Second maxillae. Elongated and tapering.

Maxillipedes. Basal article short and stout with cushions of spines disposed much as in *L. scyllicola* and bearing a large mammillated hook which does not however curve upwards. Terminal article long and slender, apparently without cushions of spines, and terminating in a claw with three small accessory claws at its base; a mammillated spine on its convex (upper in Fig. 5) edge, not similarly situated to the specific spine of *L. globosa*.

Specific characters ♀. Cephalothorax in same straight line as trunk from which it is separated by an exceedingly narrow constriction. Trunk elongated, broadest in the middle and dilated anteriorly. Antenna rounded with the terminal article of the exopodite spherical. Maxillipedes with stout basal article, bearing a mammillated spine which does not curve upwards; terminal article with a mammillated spine on the convex edge. Host: *Scyllium bivium*. Type specimen deposited in the British Museum (4 slides).

(*MS. received for publication* 20. VIII. 1929.—Ed.)

ERRATA. VOL. XX.

Pp. 183–4. The specific names of the parasite and the host should be transposed.

P. 277. Legend to Fig. 5, for *dubius*, read *bicaudatus*.

P. 278. Three lines from bottom of page, for ♂♂, read ♀♀.

A DESCRIPTION OF THE MALE OF *BRACHIELLA MERLUCCII*.

By W. H. LEIGH-SHARPE, M.Sc. (Lond.).

(With 1 Text-figure.)

Material. On 28. v. 1928 two females of *Brachiella merluccii* Bassett-Smith, 1896 were taken by the author from the tips of the gill-rakers of a single

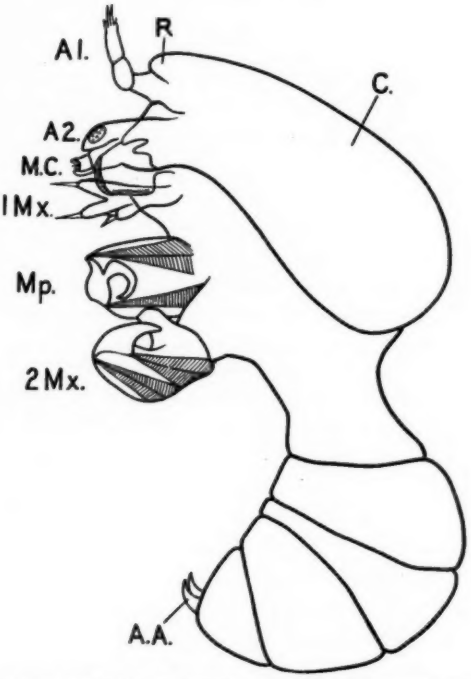

Fig. 1. *Brachiella merluccii* ♂ in left lateral aspect. *C.* = carapace; *R.* = rostrum; *A.* 1. = antennule; *A.* 2. = antenna; *M.C.* = mouth cone; 1 *Mx.* = first maxilla; *Mp.* = maxillipede; 2 *Mx.* = second maxilla; *A.A.* = abdominal appendages.

Merluccius vulgaris at Plymouth. One of these was accompanied by a male upon the cephalothorax. The female is generally well known, but there is no complete account of the appendages of the male.

Body. The general outline of the body can best be seen in Fig. 1. The males of the genus *Brachiella* differ considerably in contour, and that of

B. merluccii more particularly resembles the typical form as represented by *B. thynni* rather than that of *B. obesa* which I have previously described (1928). The head is at right angles to the trunk to which it is connected by a narrow neck. The dorsal carapace is large; there is a rostrum. Mouth cone large and prominent. Posterior to the neck or "waist" the trunk is demarcated into 3 distinct segments as is usual (not 5 as stated by Bassett-Smith), followed by an abdomen bearing a pair of small and simple abdominal appendages.

The **Appendages**, all paired, are:

Antennules (Fig. 1, *A.* 1) three-articled. The terminal article is not the smallest and ends in four setae, two long and two short.

Antennae (Fig. 1, *A.* 2) biramous, stout. The endopodite small and smoothly rounded; the exopodite two-articled and rounded.

First maxillae (Fig. 1, 1 *Mx.*), the endopodite is bipartite, each ramus ending in a stout spine, as also does the unjointed exopodite.

Maxillipedes (Fig. 1, *Mp.*) stout, tipped with powerful claws which shut down against a projection.

Second maxillae (Fig. 1, 2 *Mx.*) about the same size as the maxillipedes which they resemble. The terminal claw is, contrary to the usual condition, larger and stronger than that of the maxillipede.

REFERENCES.

Bassett-Smith (1896). Notes on the parasitic Copepoda of fish obtained at Plymouth with descriptions of new species. *Ann. and Mag. Nat. Hist.* Ser. 6, **18**, 14.

Leigh-Sharpe (1928). *Brachiella obesa*...with a description of the male. *Parasitology*, **20**, 25.

NOTE.

On 18. v. 1929 one female specimen of *Charopinus ramosus* Krøyer was taken by the author from the gills of *Raia clavata* on the Mewstone trawling grounds, Plymouth. This is the first record of its capture in English waters.

(*MS. received for publication* 20. viii. 1929.—Ed.)